PROCEEDINGS
OF THE
NATIONAL SHELLFISHERIES ASSOCIATION

Annual Meeting

Atlantic City, New Jersey August 21-24, 1950

NATIONAL SHELLFISHERIES ASSOCIATION

1950

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James B. Engle

Committee to survey regulatory or legislative powers to control the introduction of non-indigenous species of oysters.

Paul S. Galtsoff, chairman
James N. McConnell
David H. Wallace

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RESOLUTION

WHEREAS experience has shown that dangerous pests have in the past been introduced with the importation of foreign shellfish; and

WHEREAS these imported pests, freed from the natural curbs which hold them in check in their original home, have in several instances increased greatly in size and in abundance; and

WHEREAS progress in the shellfish industry requires that research on non-indigenous species of shellfish continue without undue restriction; and

WHEREAS action by individual states has already led to a diversity of laws, certain of which prevent the importation of non-indigenous shellfish for scientific study, while inaction by neighboring states leaves the industry vulnerable: Therefore be it

RESOLVED, That the National Shellfisheries Association in convention assembled recognizes the grave potential dangers to the industry of importation of such pests; and be it

RESOLVED further, That this Association urgently requests the Atlantic States and Gulf States Marine Fisheries Commission and Pacific Marine Fisheries Commission to undertake promptly a study of the problem to the end that appropriate action may be taken in all of the shellfish producing states to afford maximum protection to the industry, while leaving competent research laboratories free to continue such studies on non-indigenous species as may hold promise of yielding desirable results.

Thurlow C. Nelson, Chairman

David H. Wallacee

A. F. Chestnut

Unanimously adopted at the Joint Annual Convention of The Oyster Growers & Dealers Association, The Oyster Institute, and the National Shellfisheries Association, in Atlantic City, New Jersey, on August 24, 1950.

TREASURER'S REPORT

NATIONAL SHELLFISHERIES ASSOCIATION

June 1, 1949 - August 18, 1950

Cash on hand and in County Trust Co., Annapolis, June 1, 1949		$ 158.65
Receipts:		
Membership dues June 1, 1949 - June 1, 1950...		101.00
Total		$ 259.65
Cash on hand - County Trust Co., Annapolis June 1, 1950		$ 259.65
Receipts:		
Membership dues, June 1, 1950 - August 18,1950		$ 66.00
Total		$ 325.65
Disbursements - June 1, 1950 - August 18, 1950		
Refund - membership dues H. J. Heinz	$2.00	
Bank charges	1.04..	$ 3.04
Cash on hand - County Trust Co., Annapolis, August 18, 1950		$ 322.61

Respectfully submitted

David H. Wallace
Treasurer

Audited by:

INTRODUCTION OF NON INDIGENOUS SPECIES OF OYSTERS

Report of the Committee submitted to the National Shellfisheries Association at the Annual Convention, August 22-24, 1950, at Atlantic City

Introduction of any foreign plant or animal presents a serious problem which requires careful study and consideration before final, and frequently irrevocable, action is taken.

It is true that there are many useful plants and animals which were introduced from foreign countries. Their cultivation and propagation materially contributed to the progress of American agriculture. Yet it is equally true that many undesirable pests were unwittingly brought in to our continent, or were introduced with the valuable plants and animals as their parasites or commensals. Suffice to mention the water hyacinth, the spread of which, in the rivers and ponds of the southern states, interferes with navigation and fishing, and the European carp which proved troublesome and undesirable in the American habitat, while in Europe the fish is highly esteemed for its meat, and is extensively propagated in ponds. The combat of any pest presents great difficulties, for control operations are, as a rule, expensive and frequently ineffective.

The difficulties arising from the introduction of a non indigenous species are many. The introduced organism may compete with the native forms, and in a course of years, may replace them. An example of such a case is the introduction of the Portuguese oyster, Gryphaea angulata, to the Atlantic coast of southern France where it gradually replaced the native, and more desirable, Ostrea edulis.

Various pests and destructive enemies of native fauna and flora may be introduced with the foreign species. The spread of slipper shell, Crepidula, on the West Coast of this country and in Europe, the introduction of oyster drill, Urosalpinx cinerea, in England and Europe, and the spread of the Japanese conch, Tritonalia japonica, in the state of Washington are well-known examples of this situation.

Species which, in its native country is harmless, may become highly destructive in a new environment where its propagation is not checked by natural enemies. Examples of such cases are numerous. The most spectacular ones are the spread of the giant land snail, Achatina fulica, a native species of East Africa which, more than a hundred years ago, was introduced to some of the islands of the South Pacific, and in recent years, has spread through the Dutch East Indies, Phillippines, Mariannas, and Caroline Islands, and through mail shipment reached Hawaii in 1938. Vast destruction of vegetables and decorative plants marked the spread of this voracious snail.

planting of oysters from other areas whenever, in the opinion of a state official in charge of fisheries, such importation might be harmvul.

The States of Maine, New Hampshire, Massachusetts, Rhode Island, Pennsylvania, Delaware, Georgia, and Mississippi have no laws restricting or regulating, in any way, the importation and planting of foreign shellfish.

Among the Federal Laws relating to the protection of wildlife, the so-called Lacey Act (May 25, 1900, 31 Stat. 187-18 U.S.C. 395), authorizes the Secretary of Agriculture to regulate the "introduction of foreign birds or animals" in localities where they have not heretofore existed. With reference to the words "birds or animals", the meaning of the phraseology of the Lacey Act has not been extended to include fish or any aquatic invertebrate.

The U. S. Public Health Service exercises supervision over the importation of species of mollusks which might be concerned with the transmission of human disease. This refers primarily to the exotic species of fresh water snails some of which are known to be intermediate hosts of Trematodes.

According to the incomplete information the Committee was able to procure, at present, foreign species of oysters and clams have been introduced in the following localities: A few bushels of seed of the Japanese oyster, O. gigas, were planted in Barnstable Bay on Cape Cod by the Woods Hole Oceanographic Institution. The oysters are growing very rapidly and have reached six inches in length. A small number of the European oyster, Ostrea edulis, was planted by V. L. Loosanoff in Maine waters and attempts were made, by private persons, to introduce some of the Pacific clams including the giant Geoduck clam into Maine waters.

In the past, plantings of the Japanese oyster were attempted, on a small scale, in Louisiana, Alabama, and North Carolina. So far, the Japanese oyster has not established itself in the Atlantic and Gulf waters, and there is no evidence that Japanese conch, or other enemies associated with the Japanese oyster beds, were brought to this coast.

Continuous attempts to plant Japanese or other foreign species of oysters in the coastal areas of the Atlantic and Gulf States may eventually result in the establishment of foreign shellfish in these waters and the displacement of the native eastern oyster. In view of the serious consequences to the oyster industry that may result in a haphazard introduction of exotic species, we propose that a committee be appointed to study the problem from various angles. We further propose that the National Shellfisheries Association place itself on record as having recommended the adoption, by all Atlantic and

Gulf States, an uniform legislation for the control of the importation and planting of non indigenous shellfish in the waters under their respective jurisdiction.

Respectfully submitted,

James N. McConnell, Member

David H. Wallace, Member

Paul S. Galtsoff, Chairman

"On the functions of the mantle, gills, and palps in feeding of the oyster with especial reference to their operation in turbid waters."

Thurlow C. Nelson, Ph.D., D.Sc.,

Professor of Zoology, Rutgers University.
Biologist, New Jersey Division of Shellfisheries, State Dept. of Conservation and Economic Development.

A problem which has interested both the practical oyster grower and the scientist for many years is whether oysters are able to feed in muddy waters or in the presence of high concentrations of food organisms. Time will not permit a review of the conclusions presented during the past 34 years since the famous controversy between the late Doctors Grave and Kellogg in 1916.

From the position taken by these two outstanding zoologists one is forced to conclude that oysters will starve to death in water heavily charged with food organisms or with dirt. In 1921, however, I showed that oysters would continue to feed in water bearing as high as 0.4 gram, dry weight, of suspended matter per liter, which over most of the oyster's range in America would be classed as muddy water. In 1923 I published a summary of our observations on feeding of the oyster under natural conditions to date in which it is concluded: "4. The rate of filtration of water during any given period of time, as deduced by the rapidity and extent of ejections of accumulated sediment from the mantle cavity, may vary widely independently of the temperature and the turbidity of the water."

"5. Oysters do not necessarily feed at all times when water containing food particles is passing over the gills. Relatively little food is taken on the ebb tide and during the latter part of the night and early morning."

The issue was again brought to the fore in 1947 by the work of Loosanoff and Engle who in supporting the conclusions of Kellogg '15 and '16 showed the harmful effects of heavy concentrations of food organisms. Since these authors further proved that clear filtrates from the cultures of such food organisms likewise caused reduction or stoppage of feeding, it is highly probable that their results were due to excretions from these food organisms which the oysters found objectionable. The role of such excretions of "external metabolites" in the economy of the sea has been ably dealt with by Lucas, C. E. '47. The difficulties experienced in growing and fattening oysters in Great South Bay in recent years may well be due to similar excretions by the algal "small form" which occurs there at times so abundantly.

It is sufficient to state here that oysters can feed in nature in waters so muddy that a white saucer disappears 8 inches below the surface, and in concentrations of organisms in excess of two million in a quart of water during a heavy swarming of dinoflagellates; microscopic algae which are of great importance as food of oysters. Failure of oysters to feed and their final death in the presence of high concentrations of organisms in the laboratory is due probably to accumulation of their excretions, or so-called "external metabolites," in the water. The important point for us is that oysters will continue to feed actively in the densest populations of organisms which occur in nature provided these are not in themselves poisonous, as in the case of the red tide off the Florida Coast and elsewhere.

IF YOU WERE AN OYSTER

Our problem today is to show how the oyster is able to continue feeding in spite of high turbidity or in the presence of a superabundance of food organisms. Will you, during the next quarter of an hour extend your imagination to the utmost and will each of you picture yourself as an oyster. You live in an atmosphere of continuous dust storms often of great intensity. Your food floats in the air about you and to obtain it nature has given you four pairs of hands extending almost the full length of your body and with fingers about two feet long. The tips of your fingers are joined, while along each finger there run three groups of little whips or cilia. One group lying on the outside of the finger pushes materials downward toward the tips. The second group are long and project sideways toward the finger on either side. They act like teeth of a comb to strain out the food and dirt. The third group of whips, very powerful, lie half way around on the sides of each finger. Their beat draws air between the fingers and expels it between your legs.

This essentially is the picture we see in the early oyster spat with but one pair of "hands," the half gill, having eight pairs of "fingers," the fill filaments. Hold your hands in front of you, finger tips joined, and picture to yourself the three groups of cilia in operation. Add to these cilia rows of mucus or slime glands which entangle the dirt and food in strings and carry them along by the beating of the cilia. Also imagine a thin strand of flesh connecting the fused tips of each pair of fingers with the pair on either side. Air is passing through between your fingers, the materials strained out are entangled in mucus and driven toward the finger tips by the beat of the cilia on the outside of each finger. On reaching the tips the strings are carried forward to your mouth by strong cilia on the fused tips.

HOW THE OYSTER FEEDS

As the material collected from the water passes over the finger-like gill filaments as they are called in the oyster, more mucus is secreted about it depending on its nature. Dirt or other irritating substance evokes much mucus secretion hence by the time it reaches the lips or palps as they are called it has become so bulky that the palps reject it. Food organisms on the contrary, stimulate little secretion of mucus hence being of smaller bulk on reaching the palps are accepted and passed through the mouth into the stomach. The separation is by no means perfect, considerable sand and dirt are accepted and passed into the stomach where they undergo still further separation in the manner to be described in a few moments by Dr. Chestnut. Considerable numbers of food organisms, on the other hand, are rejected along with the dirt and are expelled out onto the surrounding oyster bed.

Numerous scientists on finding fresh living algae and other forms eliminated from oysters have concluded that they could not be digested and hence were of no value as food to the oyster. One could with equal inaccuracy conclude from the whole grains of corn passed by man and by domestic animals that corn has no food value for them. Mature oyster larvae make the journey through the digestive tract of adult oysters emerging unharmed. There is some evidence that the good sets often observed on the shells of oysters and adjacent bottom may in part be due to just such "wayward children" pulled down from the overlying waters and given a trip through the parental gut after which experience they wander no more but promptly set!

Living food organisms entangled in mucus emerging from the gut of the oyster or rejected from the palps on being thrown out onto the bottom find conditions suitable and continue to reproduce, forming additional food right on the bottom. As a result a barren bottom planted with oysters rapidly gains in food resources, worms and many other brackish water animals move in to feed on the bountiful "crumbs" that are continually falling from the oysters' "table" as it were. What fisherman has not learned of the superior fishing on an oyster bed and how quickly this deteriorates after the oysters are harvested from the ground. As applied to the oyster itself this food rejected along with the dirt fulfills in abundant measure the biblical assurance: "Cast your bread upon the water and it shall return to you many fold."

Slides were shown to illustrate the development from the simple single half gill of the early spat in which the gill filaments are joined to each other only at the tips, to the four half gills or demibranchs of the adult oyster. As development proceeds the filaments become joined to each other along their entire length leaving only minute pores, the ostia, as openings between them through which water passes into the interior of

the gill. These pores are surrounded by a thin, blood filled membrane which like the diaphragm of a camera can be enlarged or constricted to vary the size of the opening enclosed within it.

Within the gill are vertical blood vessels adjacent to each principal filament. These vessels pulsate driving the blood through them thus serving as accessory hearts. When touched even by small particles passing through the ostia the vessels contract more strongly forcing blood out into the membrane surrounding each ostium and causing the opening to be constricted. At the same time the gill filaments roll more closely together as one might depress an accordion. This brings the straining cilia at the edges of the filaments into closer contact, thus straining out the particles which were formerly passing through.

An oyster, from which one shell has been carefully removed and kept for several days in clear water, will illustrate this very clearly. Fine powder such as starch, carmine, or minute food organisms applied to the surface of the gill will pass through into the interior. After a few moments the ostia are seen to close partially, while the gill filaments are rolled closer together, accordion-like, thus bringing their straining cilia into proximity. Now the materials no longer pass through the ostia but are caught on the gills and carried toward the mouth.

One further feature of the gill must be described before its function in turbid waters can be understood. In the depth of the groove between each pair of folds lies a larger or principal filament. On this filament the cilia of the frontal area beat toward the base of the filament, not toward its tip. Materials gathered by the principal filaments pass into a food collecting furrow at the base of the gill in which they are carried toward the mouth. Such food strings reach the palp high up near its base and hence have a much better chance of reaching the stomach.

The structure and operation of the inner faces of the palps are very complex and time will not permit us to consider the details here. In brief the surface represents a series of deep grooves between folds running at right angles to the long axis of the palp. Folds and grooves are heavily ciliated and supplied with mucus glands. Food organisms which evoke little mucus secretion are passed from ridge to ridge until they finally reach the mouth. Dirt and objectionable material if not pushed off from the gills soon acquire such bulk in their passage between the palps that they are consigned to outgoing ciliary currents and so are passed to the lower palp margin and rejected. Much more selection takes place on the palps of the Portuguese oyster, Crassostrea angulata, than on those of the European oyster, Ostrea edulis, as illustrated by their great difference in size.

Ostrea edulis, feeds very largely with its principal filaments, hence it is at a decided disadvantage in turbid waters. The conclusion in 1926 of Professor C. M. Yonge now of Glasgow University, Scotland, that oysters feed with maximum efficiency when the water is relatively clear is certainly true of the European oyster on which he made his outstanding studies. Ten years later, Yonge '36, he published the most exhaustive study yet to appear on the adjustments which have been made by bivalve molluscs to increased turbidity in the environment. This study, "The evolution of the swimming habit in the Lamellibranchia," presents strong evidence that the swimming habit of such bivalves as the scallop, Pecten, arose through greater development of the muscular mechanisms for driving accumulated mud from the gill chamber of the mollusc. My paper of '38 confirmed and extended his findings as related to three species of oyster, European, American (virginica) and Portuguese.

American oysters grown in clear waters may also suffer considerable mortality when moved to waters of higher turbidity. A bushel of oysters from the very clear waters of Gardiner's Bay, Long Island, transplanted to the formerly highly turbid waters by our Cape May Laboratory on Delaware Bay several years ago suffered approximately a 50 per cent mortality within a month. In each oyster examined after death the gills were literally choked with mud. The oysters were in trays above the bottom and beside them were native Cape May oysters growing actively, putting on new shell and fattening up with stored glycogen. After approximately a month the surviving Gardiner's Bay oysters also began to grow and fatten.

Analysed biologically this simple experiment illustrates the fact that in any particular environment, muddy water for example, oysters found there are those which have been selected by nature through their ability to adapt themselves to the conditions and to survive. Moved to a new and different environment, they are at first at a disadvantage. Those which are able to adapt themselves to the changed conditions do so; the others eventually die. The same is true when conditions in an oyster producing area change* as they did in Barnegat Bay, New

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*This footnote is added in December following the great storm of November 25, 1950. Heavy losses of oysters were suffered in the Long Island Sound area as a result of this storm. In a letter dated December 27 Dr. Loosanoff states:

"However, the effect of the storm on the oysters that survived is still very obvious." (Note more than a month later) "Apparently the beating they took resulted in a great loss of their vitality..." "The oysters brought to the laboratory after the storm showed poor growth, lack of feeding, etc." In view of the relative clearness of Long Island Sound waters in normal times and the effect of moving Gardner's Bay oysters to Delaware Bay, it would be of interest to determine whether the harmful

effects observed might have been due at least in part to unusually high turbidities generated by the storm. Great numbers of oysters are reported to have been buried; those which were not covered must still have had a tough fight against high turbidities for which they were unprepared either through environmental selection or through heredity.

Jersey, during the nineteen thirties, and as conditions now seem to be changing in Great South Bay, Long Island. Experience shows that younger oysters are better able to adapt to such changes than older ones and they make such adjustments more rapidly. Our own observations indicate that oysters up to two years of age are more adaptable than those of greater age.

Some conclusions which the practical oyster grower can draw from the above are as follows:

(1). In general move oysters when they are as young as can safely be transplanted. Dr. Chestnut and I had excellent success moving the dense sets of the Cape May shores of Delaware Bay within ten days after attachment. Proof of this success is shown in an exhibit in the adjacent room. Where the set is so heavy that some of the young oysters are eliminated by their neighbors it follows that in general those which survive are those best suited to that area. If one waits until this elimination has occurred on the setting grounds the survivors are those best suited to that environment, which may be quite different from the conditions prevailing on the growing grounds where your oysters are matured.

(2). Keep more and better records, and in a book, not in your head. Every scientist called in to investigate possible causes of mortality of oysters has been forced to start almost or quite from zero in so far as the previous history of those oysters is concerned. He is dependent solely upon the memory of the planter and of his associates. Do not misunderstand me; in my experience of some 40 years I have found that most oyster growers are keen observers with good memories. Often they have pointed out to me things which I have overlooked. But memories are not infallible; keep a good log book in your own plant and see that every boat captain keeps one too, and up to date with notes written at the time or as soon as possible thereafter. Good detailed logs kept by clear sighted careful observers could soon become a gold mine of information to the scientists upon whom you must ultimately depend when things go wrong.

Make a turbidity disc; a small dinner plate or large saucer will do, lowered on a calibrated line till it just disappears; then note the depth on the line. Better, get the shop to cut you an 8 inch circle of sheet iron or copper and paint it in alternate quadrants of black and white, fasten a weight below it, a line calibrated in feet above it. Lower it from

the sunny side of the boat, note the depth at which it disappears. Record this together with the hour, clearness of the sky, roughness of the water.

Collect salinity samples especially at high and at low water and during times of excessive dry or wet weather. Ordinary pop bottles can be used if fitted with good tight stoppers and delivered promptly for analysis. Most of the maritime states now have marine laboratories the staffs of which will be glad to assist you. Remember, you are many, you are out on the grounds at all seasons and in all weathers. You are in a position to obtain much information of value to your scientific friends as well as to yourself.

(3). Keep accurate records of the volume of shucked oysters obtained from the various beds from year to year. Keep track of unusual storms and note whether volume falls off thereafter. Be sure to take salinity samples as soon as possible after such storms, for frequently they are accompanied by greater ingress of sea water which shrinks the oysters through withdrawal of water from their meats. If you are using some muddy bottoms, watch carefully for signs of mudding of the oysters and determine the advisable duration of time to leave them on such bottoms. Often early marked improvement of oysters from superior food conditions here may be obliterated through subsequent interference with feeding as the oysters sink into the mud.

(4). Finally, muddy water in itself is usually not harmful to oysters provided it does not deposit mud in too large amounts or too rapidly about the oysters. When mud rises above the bills of the oysters in such quantities as to interfere with feeding, or when by cutting off oxygen it produces large amounts of hydrogen sulphide, oysters may be suffocated, as in the severe Polydora or mudworm invasion of Maurice River Cove in the mid thirties.

Mud often contains much that is of value to the oyster. When mixed with sand it prevents it from shifting, binding it together to form some of our best oyster bottoms. Chemical and biological changes going on in mud, similar in many ways to those in the soil of a good farm, produce much of the food responsible for some of our oysters of most distinctive flavor.

Dr. Caswell Grave '12 in his "Manual of Oyster Culture in Maryland," a work which deserves reading and rereading by every research worker on oysters, was one of the first to emphasize the importance of the bottom to the food of oysters. Twenty-four pages of this excellent report are devoted to the food of the oyster and the factors favoring its increase or decrease, with emphasis upon the bottom. He attributes the superior flavor of Lynn Haven oysters to the nature of its muddy bottom. In 1921 we reemphasized the importance of bottom diatoms to

the food of the oyster estimating as many as 52 million diatoms on the shells of a single oyster 4 x 3 inches in size. In 1947 we again stressed the importance of the bottom showing the heavy films of diatoms occurring on the Cape May flats.

The great majority of studies carried on in oyster bearing areas have dealt with the water flowing over the beds. These studies are important, and we could ill afford any decrease in their number. Rather do we need considerably more of such investigations. Of equal importance, however, are companion studies of the bottom, of the contributions to it from the land, from the sea and from the animals and plants which live thereon. Dr. ZoBell in his most valuable book on "Marine Microbiology," 1946, devotes two of the 18 chapters to bottom deposits and to the activities of the microorganisms contained therein. It is a splendid beginning, but we need far more research in this field. In our knowledge of this all important problem we are about where the farmer was a half century ago, but as he and the world at large have reaped vast benefits from the researches in soil science, so may you and we gain much in the future as we learn more about the relation of the oyster to the grounds on which it lies.

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Studies on the digestive system of the oyster.

A. F. Chestnut

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Many papers presented at the annual National Shellfisheries Association during the 1930's were on the role of oysters in human nutrition. These papers by Drs. Pease, Remington, Coulson, Whipple and others discussed vitamin values, iodine content, oysters and anemia and nutritional values. One result of these studies was applied in advertising to promote greater sales of oysters.

Since 1942 a number of papers have been presented at the annual meetings on the basic problem of the oysters' nutrition or "what do oysters eat?". Oyster growers and shucking house operators are well aware of the fluctuations that occur in a single season and from year to year in the yield of oyster meats per bushel measure. Some years the yield from the same locality may be as low as 4 pints of meats per bushel and in other seasons the yield may be as high as 9 or 10 pints per bushel.

During the past fifty years many scientific studies in this country and abroad have shown that the chemical and physical environment closely associated with the food or plankton in the water may exert a great influence on the condition and yield of meats. The literature is too voluminous to review in this brief discussion but various theories have resulted from the studies on the nature of the food of oysters and other lamellibranchs. Some investigators have concluded that oysters feed exclusively on plant-like organisms; others believe that animal forms are utilized as readily as plant forms; and still others maintain that living forms play a minor role in contributing to the actual food supply.

The original intent of this study was to follow the digestion of various foodstuffs by the oyster and thus determine the process involved in increasing the yield of meat. In the course of study it was found that descriptions of the internal anatomy of Ostrea virginica were lacking and needed to understand the processes of feeding. The internal anatomy was found to differ from that described for the European oyster (Ostrea edulis) by Yonge (1926), for the Portuguese oyster (Ostrea angulata) by Leenhardt (1926) and for the Bombay oyster (Ostrea cucullata) by Awati (1931). This is not surprising for other investigators have shown differences in the gross anatomy in the gills and other structures, in reproduction and in physiology between various species. (Orton, 1928, Elsey 1935, Nelson 1938, Galtsoff, 1932).

Description of Stomach:

The sorting mechanism of the gills and palps has been described in the previous paper by Prof. Thurlow C. Nelson. The various food and other materials passing over the gills and palps enter the mouth and pass to the stomach through a relatively short oesophagus. At first glance the stomach appears to be without definite shape but gelatin casts show that several definite ridges are present. Seven openings are found leading into or from the stomach lumen. At the anterior end of the stomach is the opening of the oesophagus and at the posterior end is the combined opening of the style sac and intestine. There are four openings connecting the stomach with the digestive diverticula, two in the antero-ventral region, one in the posterior half of the stomach on the lower left wall and the other nearly opposite on the lower right wall. At the anterior lower left corner of the stomach is the opening leading to the food sorting caecum.

The most conspicuous structure is the ridge or typhlosole along the floor of the stomach. The anterior end of the typhlosole passes into the food sorting caecum and the posterior end turns dorsally to enter the intestine. On the right wall are three ridges lying in a dorso-ventral position. These ridges end slightly below the mid-section of the right wall at a longitudinal ridge which overhangs the posterior right duct, and continues anterior to form a transverse ridge under the oesophagus. On the left wall is one large convoluted ridge extending from the roof of the stomach ventral to the mid-section of the left wall.

The food sorting caecum is an outpocketing of the stomach originating at the left anterior portion of the stomach and extends posteriorly between the mantle and stomach, then curves to the right under the floor of the stomach and continues in a coiled fashion to end under the stomach after describing one and one-quarter turns.

An irregular, two-lobed translucent gastric shield is located along the dorsal portion of the left wall, extending to the dorsal surface of the stomach. In animals which have been actively pumping water, a crystalline style extends from the opening of the style sac and projects across the stomach to bear against the gastric shield.

Course of Ciliary Paths in the Stomach:

The stomach was laid open by dorsal, ventral and lateral cuts to determine the paths followed by the particles entering the stomach. Mixtures of carmine and various grades of carborundum were used for inert materials and suspensions of algal cells (Navicula, Platymonas, Chlorella, zoospores of Ulva), and starch grains were used as food material.

Particles that enter the mouth are carried posteriorly along the oesophagus. At the inner edge of the oesophagus, near the stomach, the ciliary beat is changed and the particles are carried toward the left and come under the influence of the cilia of the large convoluted ridge of the left wall which beat dorsally toward the gastric shield. Heavy particles that may pass directly into the stomach from the oesophagus come under the influence of the cilia of the transverse ridge under the oesophagus and are carried toward the right wall where the ciliary beat is toward the gastric shield.

In the posterior half of the stomach the ciliary beat is posterior and ventrad so that particles are carried in two directions, toward the posterior right duct of the digestive diverticula or end up in the ventral groove along the floor of the stomach and are carried into the intestine.

The ciliary beat on the typhlosole or ridge on the floor of the stomach is anterior, carrying particles into the food sorting caecum. Along the right side of the ridge is a deep groove or furrow with powerful ciliary beat toward the posterior, carrying particles from the stomach into the intestine. Food is consolidated in the intestine and carried along to the rectum and anus.

Heavy particles that enter the stomach cause much mucous to be secreted and strings are formed that are generally swept across the weaker ciliary paths by the beat of the stronger tracts. Perhaps the most powerful ciliary beat is that of the ventral groove. Another powerful force in changing the normal course that particles may follow is the crystalline style which twirls around as it projects across the stomach. Mucous strings are wound about the head of the style and particles are diverted from their normal course.

The role of phagocytes in feeding:

Throughout the oyster, in the tissues, blood vessels, in the lumen of the stomach and intestine, on the gills and in the mucous masses are found numerous wandering phagocytic cells. The ability of these cells to ingest and digest various foodstuffs has been shown by Yonge (1926) in Ostrea edulis, and were investigated in these studies. Oysters were fed starch grains, algal cells and yeast cells, in vivo, and stomach contents were examined at hourly intervals to determine how rapidly the materials were ingested. Within an hour diatoms and starch grains were found engulfed and plasmolysis of algal cells occurred within two hours.

Studies in vitro showed that ingestion may occur within 30 to 45 minutes. Complete disintegration of algal cells takes place within two and three hours. Empty diatom tests and reddish-brown granules, presumably the undigested residues, were frequently seen being egested by the phagocytic cells. Yeast cells

stained with Congo red showed a color change from red to purple indicating an increase in acidity within the cells. India ink, carmine and carbon particles were readily ingested and within an hour after ingestion the particles were passed out.

Injections of food particles under the surface epithelium resulted in concentrations of phagocytes in the immediate area with many of the particles being ingested. Some particles were eventually seen passed out in the fecal strings.

Digestive enzymes:

Quantitative and qualitative studies were made of the hydrolytic enzymes in various extracts. The extracts of the crystalline style contained strong amylytic enzymes that readily reduce starch to sugars. Lipolytic and proteolytic enzymes were found in the extracts of the digestice diverticula and in the mucous masses containing phagocytes. The stomach contents were found to contain only an amylytic activity.

Summary:

In the process of feeding a rigorous selection of particles occurs on the gills and palps before they reach the mouth. Within the stomach an additional sorting may take place by ciliary action, mucous secretion and within the food sorting caecum.

In the living animal the paths that particles follow may vary considerably depending upon a number of variable factors. The amount of inert particles, concentration of food organisms, rate of mucous secretion and the influence of the crystalline style all have an effect upon course particles will follow.

These studies described are far from complete, but they do suggest that the food of such forms as oysters need not be limited to plant or animal forms exclusively. Any organism, whether plant or animal or a constituent part, that is capable of being ingested and digested would be of importance in contributing to the food supply.

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Variation in Salinity and Its Relation to the Florida Oyster

PART ONE:

Salinity Variations in Apalachicola Bay

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Several of the estuaries of Florida are not well protected. Water from the rivers many times enters the Gulf and Atlantic abruptly and without an opportunity to become uniformly mixed.

Much of the present-day oyster industry of Florida is centered in estuaries which do not provide a constant and uniform salinity. Variations exist not only seasonally, but daily and tidally.

Biologists and producers alike would be benefitted by a knowledge of the magnitude of variation which oysters can tolerate in Florida's sub-tropical waters, and what degree of variation can be considered optimal for reproduction, deposition of glycogen, maximum growth, etc. The present study was carried out to establish, if possible, the critical values for salinity variations as they affect oyster well-being.

Procedure

Salinity studies (by hydrometer) were made on a series of habitats ranging from the poorest to the best with regards to commercial production. Density was obtained for other stations frequently, and there were several sets of paired readings run on consecutive high and low tides. In some instances salinity tests were run on bottom and top samples hourly for a 24 hour period. The studies were carried on for a period of eighteen months (February, 1949, to July, 1950). Where practical, frequent correlative glycogen studies were made on oysters from habitats under observation.

Results

As was expected, substantial variations in salinity were observed seasonally and weekly. Surprising were the large variations found intertidally and hourly in certain cases.

Seasonal salinity variation (as standard deviation) and oyster condition are shown together with eighteen month ranges of salinity for various stations in Apalachicola Bay in Table I. Maximum weekly ranges of salinity for all stations and maximum ranges intertidally are presented.

A summary of all data gathered is presented in Table I.

Discussion

The oysters of Apalachicola Bay, from the standpoint of salinity variation, live in a rigorous habitat. During their development to marketable size they are exposed to a range of salinity extending from fresh water, or nearly so, to ten or more parts per thousand more than is found in ocean water.

More frequent changes of salinity of substantial magnitude are endured. Tidally, variations of 8-10 parts per thousand are not uncommon. In one instance (Cat Point) a variation of 15 °/oo was observed in an area of high productivity.

Occasional weekly variations of approximately 25 °/oo do not seem to interfere with the development to marketable size and quality. Indian Pass consistently had the best oysters of the Bay during the winter of 1949-1950, yet the average weekly salinity change for that station was 5.9 °/oo (Maximum 24.3 °/oo).

A correlation between magnitude of salinity change per unit of time and oyster quality (based upon glycogen and production) does not give conclusive results. Apparently, oysters in one habitat can stand a tidal variation of 10.6 °/oo as an average and still be of marketable quality (Indian Pass). This lack of correlation is also noticed in weekly and seasonal changes.

There is some reason for believing that range of salinity variation may be a more critical factor for oyster production in Florida than the high temperatures they experience. Fast growing, high quality (ave. gly. 3.5%) oysters are found consistently in commercial quantities 154 miles north of Miami in the Indian River. The daily temperature of the water in that area during December, 1949, January, and February of 1950, averaged 24.3 °C. During the three month observation period, however, the change of salinity from day to day averaged only 1.7 °/oo. There are no appreciable tides in the area.

It was observed that the quality of the Indian River oyster was the same during the entire winter. The quality of oysters from the best area of Apalachicola Bay did not remain constant during this period although the average temperature was lower than that of the Indian River. The average water temperature during December, 1949, January, and February of 1950, was 18.9°C. for Apalachicola Bay. This figure is based upon 45 observations taken during that period at widely isolated parts of the estuary.

Summary

1. An eighteen month survey of salinity conditions has been made of Apalachicola Bay, present center of Florida's oyster industry.

2. Annual variations in salinity from fresh water to 42.5 °/oo are not only tolerated by the indigenous oysters, but commercial production is present where those conditions exist.

3. Marketable oysters in Apalachicola Bay are found in habitats which experience weekly salinity changes averaging 11 °/oo.

4. Commercial production of oysters is possible in habitats showing an average tidal salinity variance of 4.8 °/oo.

5. Growth rate studies carried on simultaneously with the salinity investigation indicate that growth under the salinity variations mentioned above is extremely rapid. One inch in length is achieved in five weeks, and 2.6 inches in 16 weeks.

6. In general, Apalachicola oysters are not of superior quality. It is suggested that the low glycogen content might be due to the great ranges of salinity to which they are exposed.

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TABLE I

Summary of salinity observations taken daily, weekly, and tidally correlated with production estimate and maximum observed glycogen. Salinity figures refer to parts per thousand.

Station	18 Months				Intertidal			Weekly				
	No. Obs.	Range	Standard Deviation	Ave. Sal.	No. Obs.	Greatest Range	Ave. Range	No. Obs.	Greatest Range	Ave. Range	Produc.	High-est Obs. Gly.
Pilot's Cove	68	11.9-42.4	7.0	24.5				20	20.5-35.6	8.3	Small	0.3
Cat Point	144	0.0-32.1	6.2	15.6	10	8.0-23.0	4.8	61	0.0-22.2	7.1	Medium	0.4
Porter's Bar	158	0.0-42.5	6.1	19.8	9	9.2-13.1	1.9	56	15.4-42.5	7.7	Heavy	1.3
Green Point	57	3.2-26.5	5.4	14.2	6	16.0-21.4	3.8	13	13,6-26.5	5.7	Heavy	1.6
Indian Pass	143	16.1-43.8	6.1	27.2	11	25.0-33.3	3.1	54	10.7-35.0	5.9	Heavy	5.6
Paradise Flat	36	4.3-32.8	9.5	16.3	4	5.4- 7.0	0.8	7	5.4-27.4	10.6	Heavy	0.3
Cabbage Lump	77	4.3-34.5	7.7	16.3	9	13.1-30.0	4.8	17	9.9-33.0	11.0	Heavy	0.5
St. Vincent's	197	2.6-34.9	8.3	22.1	14	14.1-30.2	6.6	21	10.8-34.0	12.0	None	0.9
Miller's 13	113	3.3-43.8	9.4	20.6	5	5.0-22.7	6.7	49	3.5-33.7	10.2	None	0.7
Nick's Hole	83	0.0-33.6	7.3	17.5	2	7.2-15.6	6.9	20	0.0-20.3	7.4	Small	1.7
Goose Island	33	7.0-32.7	6.5	20.6				11	10.7-26.0	4.7	None	
East Slough	28	5.9-32.9	7.1	21.6				11	11.4-24.2	4.8	None	
Govt Dock	49	4.8-42.8	7.4	22.2				37	16.7-42.8	9.2	None	

The Condition of Oysters as Measured by the Carbohydrate Cycle, The Condition Factor and the Per Cent Dry Weight

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The fact is well established that a wide range of quality in oysters exists wherever they are found, on different parts of one oyster bar as well as between distinctly separated areas. Investigators have proposed many reasons for quality differences, some of which can be listed as follows: changes in salinity and temperature, availability and assimilation of food, density of growth or planting, internal and external parasites, the presence of shell invading organisms, industrial pollution, physical transfer of oysters from one bed to another and probably others.

Oysters are valued commercially by the condition of their meats. Methods of determining the relative values are both qualitative and quantitative. To say that a creamy white turgid oyster is good is redundant when mentioned here in this company of experienced oyster dealers. Likewise, that a flaccid, gray, watery animal is poor. There is no doubt that qualitative estimates of condition are useful and valuable in the trade. The drawback here of course is in the lack of uniformity of comparison which depends on the experience and individual reactions of the observer. To remove from these estimates of condition the variability introduced by observers using qualitative methods certain techniques have been devised and applied to measure in an unbiased way some of the attributes of oyster condition quantitatively.

In the shellfish laboratory of the U. S. Fish and Wildlife Service, Annapolis, Maryland, oysters from Chesapeake Bay are examined by certain techniques to determine condition. These show the amount and seasonal cycle of glycogen accumulation, the relation of the amount of meat to the size of the shell cavity, the ratio of which is known as the condition factor, and the per cent dry weight. Each of these is an index of the condition of the oyster. Each of these also answers a somewhat different question.

The most obvious index of condition is the "fatness" attributed for the most part to the presence or absence of glycogen, an animal starch. The method of glycogen determination is a long established procedure of digestion of the meats with hot alkali, precipitation of the glycogen with alcohol, hydrolizing the glycogen to glucose and determining the amount of sugar colorimetrically. Results are expressed as percentage of glycogen in dried oyster meats. A portion of each sample of meats is dried to determine the per cent total solids.

In Chesapeake Bay, as in most places, the glycogen content of oysters follows a cyclic or seasonal pattern. In late spring a rapid reduction in glycogen takes place which is coincident with the fairly rapid development of the sex products prior to spawning. Low glycogen is reached shortly after the gonads begin the initial discharge of spawn. The low glycogen content is maintained with some fluctuation until the termination of spawning when a rapid reaccumulation of glycogen begins. The time of the drop and the recovery at the end of the summer varies to some extent from year to year. Major changes in the normal glycogen cycle, nevertheless, remain coincident with the gonad development and the spawning reaction. In 1949 the major drop in per cent glycogen occurred over the period May 5 to June 15, and the recovery over the period October 17 to November 21. The initial spawning in 1949 was about June 12. In 1950 the major drop in per cent glycogen took place two weeks later and proceeded at a slower rate. The initial spawning in 1950 was about July 3. The change in the per cent glycogen from the winter reserve to the summer minimum in 1949 was from 24% to 3%, and in the fall it returned to 35% which held through the winter. The early summer drop in 1950 reduced the winter reserve of 35% to 9%. Oysters were considerably better in 1950 than they were in 1949.

Glycogen in oysters, then, as an index of condition, has a twofold significance, one dealing with marketable values and the other with biological phenomena. The latter point in some measure influences market values in the following manner. The season of harvest, usually set by legislation, only partially encompasses the period of high glycogen reserve. At the end of the open season, April 15 in Maryland, "fat" oysters high in glycogen are still quality products. But at the beginning of the open season, September 1 in most waters of Maryland, oysters are still in a spawning condition with relatively low glycogen reserve. From the standpoint of production and conservation harvesting of oysters in September has these arguments against it: (1) spawning is still in progress, (2) quality of meats is usually poor, and (3) yield per bushel in volume of meats is low. The last two points are directly related to the low glycogen reserve. The glycogen cycle recapitulates itself annually with fair regularity. Knowing this, the conditions detrimental to production and conservation mentioned above, may be alleviated by postponing the beginning of the oyster harvest to the period in the cycle when the high glycogen reserve has been reaccumulated or after October 15. Admittedly, this presents a problem in economics in a highly competitive industry. But again, the industry in the Chesapeake area and elsewhere suffers to some extent when oysters low in glycogen, as they are in September, are placed on the market.

The role played by glycogen in the biology of the oyster is only partially known. What is evident, however, is the intimate connection glycogen has with the production of sex products. The early season drop in the glycogen reserve is

concurrent with the rapid production or maturing of the eggs and sperm in the gonads. The fall accumulation of glycogen follows the termination of spawning. To further support this relationship was an observation made during the summer of 1949. The initial spawning had slowed down and the glycogen drop had ceased. Then began a slow rise in the amount of glycogen during the middle two weeks of July. During the last week of that month the gonads increased in thickness and the glycogen simultaneously dropped rapidly to a new low. In a lesser way the mid season spawning-glycogen relation repeated the initial reaction.

A simple means of determining the volume yield of oyster meats from a measure of oysters in the shell has been known for many years. Dr. Caswell Grave in the early part of this century calculated by displacement the volume of the whole oyster in the shell and compared it with the volume of wet meats. The ratio of the meats to the whole oyster can in turn be converted to a pints per bushel yield, the index generally used by the oyster packer to classify the condition of his oysters. Grave then went one step further and compared the volume of meats to the volume of the shell cavity to eliminate the discrepancy introduced by the individual differences in the thickness of shells. On a wet basis the ratio of the meats to the shell cavity represents a condition factor for comparing quality between groups of oysters. The weakness in this method is the use of the volume of wet meats which may be bloated when exposed to fresh water. Grave pointed this out.

Dr. A. E. Hopkins in the United States and Dr. C. J. Medcof in Canada much later employed Grave's methods adjusted to the dry weight of meats as a quantitative means of comparing quality in oysters. The index called the Condition Factor is a product of this latter method.

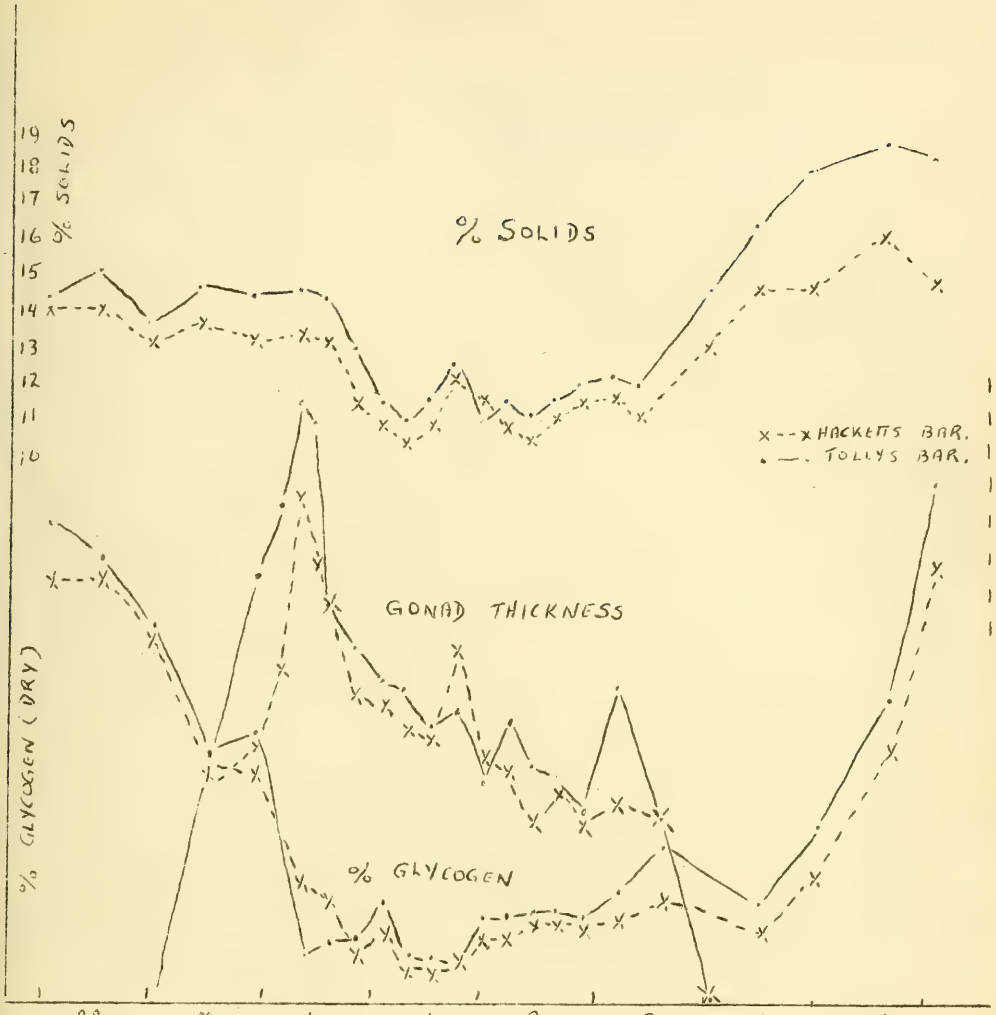
In Maryland and Virginia the Condition Factor has been used currently to indicate relative differences in the quality of oysters. The range of "2" for very poor to "16" for excellent was arrived at empirically. With this scale of quality the changes in condition of oysters was measured during the extended period of freshet in 1945 and 46. The gross appearance of the oysters did not always show the degree of "poorness" in its true light because of the bloating caused by the fresh water. When measured by the Condition Factor procedure, which excluded the excess water, the true value of the meats was indicated. In general the Condition Factor analysis has a similar cycle to that of the glycogen cycle.

The per cent dry weight of oyster meats from which can be calculated the total solids or the moisture content is utilized in the glycogen and the Condition Factor methods of determining quality. In itself it has a place in comparing oyster meats with other foods. A common measure of food value is the total

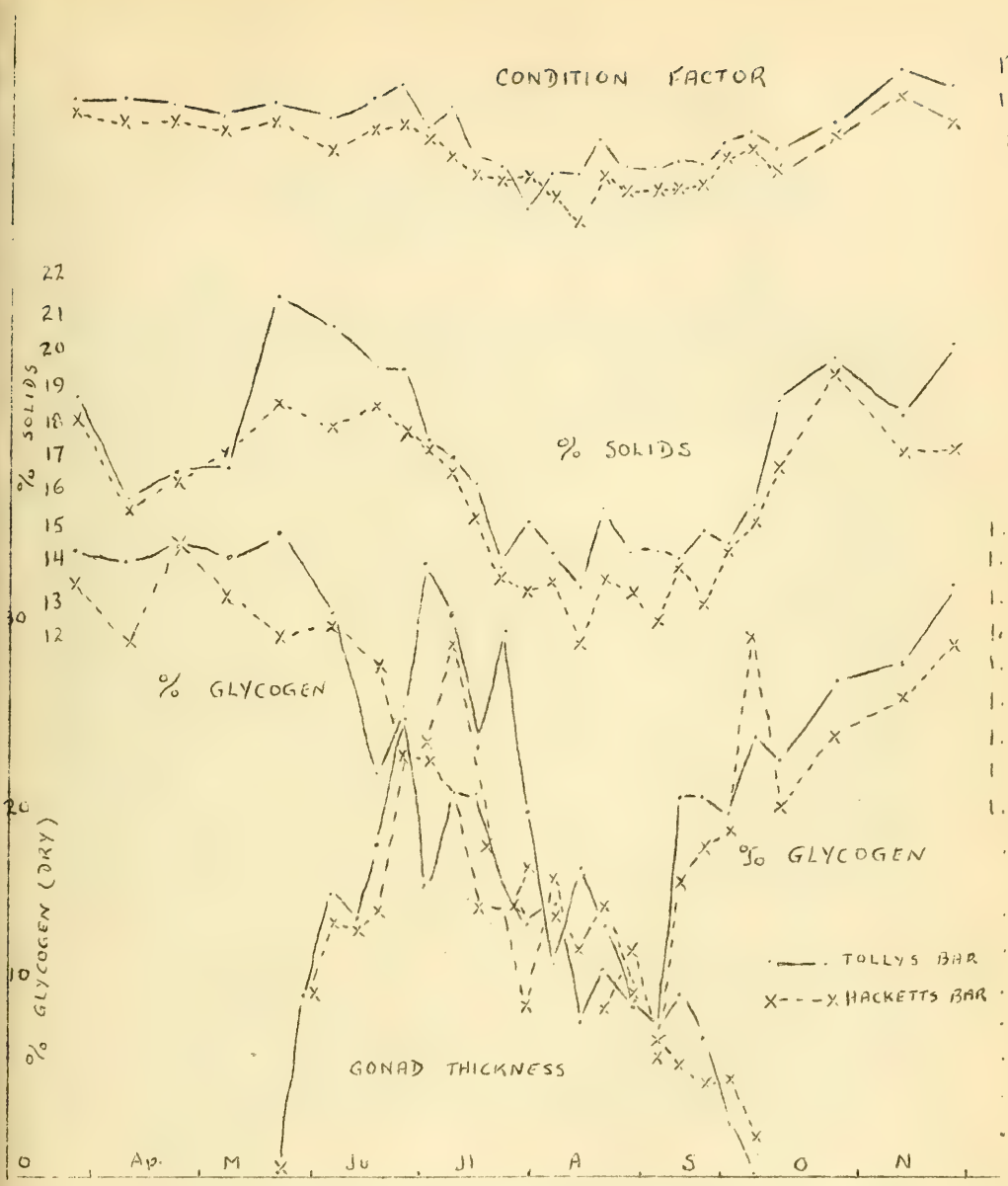
solids. With this index it is possible to relate the unit value of oyster meat to unit values of other protein foods. As in the other methods of quantitatively determining quality of oyster meats, this technique may be used to compare one group of oysters with another.

With methods that accurately and simply measure the quality of oysters, it is possible to compare the effects of changes in the environment, inherent differences in the individuals and cultivation procedures on the condition of oysters. Fundamentally it must be known what constitutes a good oyster and why. The "why" is the hurdle the oyster biologist is working with. Some of the measuring devises he may use are explained here.

Two charts.



1949. COMPARISON OF CONDITION INDEXES WITH GONAD DEVELOPMEN & SPAWNING OF OYSTERS ON TWO BARS, CHESAPEAKE BAY



1950. COMPARISON OF CONDITION INDEXES WITH GONAD DEVELOPMENT & SPAWNING OF OYSTERS ON TWO BARS, CHESAPEAKE BAY.

Shellfish Sanitation Research Program*

By

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In 1946, the Public Health Service published the first edition of its manual of Recommended Practice for the Sanitary Control of the Shellfish Industry. In the formulation of this first edition valuable assistance and guidance was obtained from discussions at meetings of the Shellfish Committees of the American Public Health Association and from meetings of the National Shellfisheries Association. This assistance has been continued through succeeding years.

Invariably, the subjects which received most attention during these discussions were concerned with bacteriological procedures, and what would constitute reasonable and practical bacteriological standards for shellfish and shellfish producing waters. A standard bacteriological procedure has since been developed, and has received universal acceptance. There remains a deficiency in fundamental knowledge of the bacteriology of shellfish. Sufficient scientific data are not available to establish such vital facts as the relative survival of coliform organisms and pathogens in sea water, the relative ratio of coliforms in the various species of shellfish growing in the same area, and the bacteriological behavior of shellfish as they proceed from the growing area to the ultimate consumer. Without such knowledge, the practicing shellfish sanitarian has no standard on which to base an intelligent opinion or interpretation of his sanitary surveys. He can not establish the bacterial density at which shellfish should be allowed for distribution and sale.

Recognition was made of this situation by the Service during the compilation of the first edition of the manual. As soon as possible after World War II, steps were undertaken to establish a research laboratory in which to determine these fundamental facts. The first step was the appointment of an Advisory Committee composed of bacteriologists, biologists, and sanitary engineers prominent in shellfish sanitation. State and Federal Agencies, as well as industry, are represented on the Committee.

The first meeting of the advisory group was held in March 1948, in Washington, D. C. It was decided that a research

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* Presented at the Annual Meeting of the National Shellfisheries Association at Atlantic City, N. J., August 22-25, 1950.

laboratory should be established at Woods Hole, Massachusetts, staffed and operated by the Public Health Service. Selection of this location was made because of the availability of extensive laboratory and library facilities, and the opportunity for technical consultation and advice at the three scientific organizations devoted exclusively to oceanographic research. Also Woods Hole is near waters of various degrees of pollution from the metropolitan communities at some distance from the station and the few isolated areas in the immediate vicinity.

The Shellfish Advisory Committee recommended the following schedule of investigations:

1. An evaluation of existing methods for the bacteriological examination of shellfish.

2. The determination of the relationship in bacteriological content between the shellfish and the overlying waters at various levels of pollution and temperature ranges.

3. The study of the relative survival of coliforms and enteric pathogens in sea water and shellfish.

4. The study of natural and artificial purification of shellfish.

Organization - The Woods Hole laboratory is a section of the Research and Development Branch of the Environmental Health Center at Cincinnati, Ohio. It also maintains a close liaison with the Shellfish Sanitation Branch of the Division of Sanitation which is responsible for the shellfish sanitation control program of the Public Health Service. Thus, the scientific findings obtained in the laboratory may be applied at the commercial level and in return, the practicing sanitarian has the aid of technical and professional advice on his field problems. With the close association of the two units, it is also possible for coordinated guidance of the shellfish advisory group.

The Woods Hole laboratory staff includes a chemist, two bacteriologists, a biological aide, and a stenographer. Laboratory facilities were first located at the Woods Hole Oceanographic Institution and work was started on problem one: the comparison of bacteriological methods, in May 1948. When sufficient information was obtained from this study, standard bacteriological procedures were formulated with these, it was possible to undertake other investigations. However, it was soon found that the allotted space at the Woods Hole Oceanographic Institution was inadequate for proper functioning of the laboratory, and negotiations were started for the acquisition of additional space. Arrangements were finally completed in May of 1950 for the use of three rooms located at the laboratories of the Fish a nd Wildlife Service at Woods Hole. The laboratory moved to the new quarters in June.

Summary of Results of Investigations

1. Comparative study of the methods for the bacteriological examination of shellfish - Laboratory work on this project has been completed, the data have been tabulated, and the final report is in preparation. Results of the study indicate that the procedure for the enumeration of coliform organisms as described in the Recommended Procedures of the American Public Health Association is practical, and could be followed with little modification. The same conclusion applies to the Winter-Sandholzer technique for the determination of enterococci.

2. Ratio of coliforms and enterococci in Shellfish and the overlying waters - In these experiments three species of shellfish were planted in a laboratory flat, and exposed to flowing sea water which had been artificially contaminated with known and controlled quantities of pollution. The pollution was supplied from a reservoir which contained a dilution, in phosphate buffered water, of the unchlorinated Imhoff tank effluent of the sewage treatment plant at Camp Edwards. The pollution reservoir was changed twice daily, and most of the experiments were continued for a period of four days.

Studies were conducted at three levels of pollution; that of clean water without added sewage; at coliform MPN approximately 400, representing moderate pollution; and at coliform MPN 1000 and above, representing heavy pollution. They have been conducted at three ranges of temperature; 0°-5°C, 8°-15°C, and approximately 20°C. This project is now nearing completion, and it is expected that results will be available by late fall.

Although tabulation of data is not complete, the results to date indicate that shellfish respond quite rapidly to changes in the bacteriological content of the overlying water, usually within twenty-four hours, and, in some instances, within eight hours. Although a definite ratio between the shellfish and the overlying waters has not yet been computed, it appears to be relatively constant at the three pollution levels studied. It has been determined that under similar conditions of pollution soft clams show the highest coliform index of the three species studied. Oysters and hard clams are of the same order, with the possibility that oysters range slightly higher.

At the end of the pollution runs clean water was allowed to flow over the flats, and the shellfish were examined at frequent intervals for a period up to seventy-two hours. Decreasing the coliform density of the water affected a corresponding drop in the coliform content of the shellfish at a rather rapid rate, the process usually attaining stabilization within a period of eight hours.

3. Lussel Studies - The Shellfish Committee, Engineering

Section of the American Public Health Association, at a meeting in Boston in 1948, petitioned the Service to include in the program of activities a study on the cause of high coliform scores obtained in mussels as received at the wholesale market. This problem has received attention as often as time permitted, and the studies will continue until a satisfactory answer has been obtained.

Part of the study has included a careful check on lots of mussels commercially dug and shipped to the market. Bacteriological examinations were conducted on mussels stored in bushel baskets for 24, 48, and 72 hours at room temperature; shipped to New Bedford and returned; and shipped to Fulton Market, New York. Up to the present time the results indicate that the increase in coliforms is primarily a function of time and temperature; but it is also dependent on the extent to which the mussels are cleaned of adhering mud, as well as care in handling after removal from the water.

4. Seasonal variation of coliforms and enterococci in a polluted area - Eel Pond Studies - Eel Pond is a small tidal area located within the village of Woods Hole. It receives a considerable amount of pollution from dwellings and semi-public places built around it, and therefore, was considered to be an ideal area for the study projected. Accordingly, samples of water have been examined from six stations, and hard clams from one station, at monthly intervals since August 1948. A sanitary survey of the area has also been made in order to correlate the bacterial findings with the locations of sources of pollution. Work on this project has proceeded to a point where sufficient information has been collected for the preparation of a final report. Definite seasonal variations in bacterial densities have been observed, with the low coming in the spring following the period of lowest water temperature. Some correlation between activity of the shellfish and bacterial content also was demonstrated; the hard clams showing a greater coliform content in the summer and less in the winter.

5. Survival of coliforms and enteric pathogens in sea water- This project was suggested by the Advisory Committee at its meeting at Woods Hole in October 1949. It is in the preliminary phase; a project outline having been compiled, and a standard technique developed.

In the first part of the study suspensions of coliforms and salmonella, both in pure culture and combined, are prepared in Berkfeldt filtered sea water. These are examined periodically to the point of extinction. Later, experiments will be undertaken in model shellfish flats, the shellfish to be supplied with water containing known concentrations of coliforms and pathogens, and the water and shellfish will be examined after various periods of time. These experiments will be conducted under different temperature conditions. It is hoped that these experiments

will result in the establishment of the rate of absorption of coliforms and pathogens by the shellfish, as well as the relative concentrations of these organisms in the shellfish and the overlying water. Rate of cleansing will be determined by supplying the flat with clean water at the end of the experiment with frequent analyses of shellfish and overlying water until the coliform content of the shellfish is the same as that of the overlying water, and pathogens are completely eliminated.

Summary - The organization and aims of the Shellfish Sanitation Section of the Environmental Health Center, have been described. The purposes of the laboratory are to conduct studies with a view to the development or improvement of bacteriological methods for the examination of shellfish waters and the collection of data leading to the development of reasonable control standards. The unit also operates in collaboration with Federal and State control agencies in practical field investigations in the sanitation of harvesting, processing and marketing of shellfish.

A brief description has been presented of several studies undertaken by the laboratory. Some have been completed, on which reports will be available in the near future.

The laboratory has to date, made an evaluation of methods for the bacteriological examination of shellfish and overlying waters and has collected sufficient data under controlled conditions to establish the relative concentration of coliforms and enterococci in the three species of shellfish and the overlying water. More specific information on the significance of this relationship will be obtained from similar studies with pathogenic organisms.

Other studies in progress include investigations of the handling and marketing of mussels and the artificial and natural purification of soft clams. Work on these projects has not yet progressed to a point where definite conclusions can be drawn, but the studies will be continued until sufficient information has been obtained.

Observations on Soft Clam Mortalities in Massachusetts

by

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Introduction

This paper is to report a mass mortality among soft clams, Mya arenaria, observed in Plum Island Sound, Essex County, Massachusetts, and to compare the occurrence with similar ones reported at other times and places along the New England coast. These mortalities may be a primary cause of the present scarcity of soft clams in Massachusetts.

Mortalities in Plum Island Sound, 1949

During the summer of 1949 we made a census of the clam population in Plum Island Sound, principally by taking two-square-foot samples. By the middle of summer it became quite obvious that clams were dying from some unknown cause. They were in various stages of decay, but in normal position in the mud, often with their rotted siphons still extended to the surface of the flat. This is the condition I am calling "mortality", in contrast to the loss of clams by predation or other causes where the clams are removed bodily.

The mortality was most obvious in a bed of planted clams in Rowley, partly because there were plenty of clams there whereas they were scarce elsewhere. On June 15, nineteen live and five rotting clams were dug from three square feet, a 21 per cent mortality. By the middle of July, 98 live and 73 dead were dug from ten square feet, a 43 per cent mortality.

At Hale's Cove, about a mile and a half farther up the Sound, the proportion of rotten clams was even greater, though not as obvious because clams were scarce. There was a fair scattering of 20 to 40 mm. clams over much of Hale's Cove, estimated at a density of one or two per square foot, but thicker in places. Forty screened samples, each two square, taken between May 24 and July 6 had 78 live and 27 dead clams, a mortality of about 26 per cent. On October 18, only five live clams and 148 dead ones were found in 115 square feet, a mortality of about 97 per cent.

Clams which we planted for farming experiments fared little better than the native stock. In this case the horseshoe crabs bodily removed 92 per cent of our planted clams, mostly within two or three weeks after planting, but of the remaining clams, 318 were recovered alive and 914 in various stages of decay, a

mortality of 69 per cent. Many of the rotting clams had grown as much as the live ones, indicating that death occurred progressively throughout the summer. The new growth was very distinct on these stunted, thick-shelled transplanted clams.

Mortalities such as I have been describing apparently were quite general in Plum Island Sound.

Areas of Low Mortality, 1949

There were also areas in Plum Island Sound notable for their lack of mortality. One of these, on Dole's Island at the mouth of the Parker River, is less than half a mile from Hale's Cove. Eight two-square-foot samples contained 273 live and only 9 dead clams on July 28, and general inspecting in October and November indicated little or no increase. This small mortality could have resulted from injuries by diggers that worked there during the summer. Other mortality-free areas were found two miles farther up the Parker River, in the Merrimack River and in Black Water Creek, a tributary to Hampton River, New Hampshire.

Past Mortalities at or near Plum Island Sound

This clam mortality is not a strange new catastrophe. It has occurred before. Dr. Galtsoff, of this Service (Unpublished report), examined the Hale's Cove area in 1946, and his description of rotting clams would do as well for 1949. One square foot sample had 12 live and 35 dead clams.

A mass mortality at Essex in 1914 was described by H. W. Nightingale (U.S. Bureau of Fisheries Economic Circular 16, 1915) and many old clam diggers along the coast can recall years when they found clams rotting in the flats.

Clam Mortalities in Other Areas

Mass mortalities have occurred over extensive and widespread areas along the Atlantic Coast in recent years. Biologists up and down the coast have witnessed them in Canada in 1932, in various isolated spots in Maine in 1949, and over the last few years in Connecticut, New York, and New Jersey.

Possible Causes of Mortality

So far, we have not determined the cause of the mortality. About all we can do at present is to narrow the possible causes down to a few of the most likely.

There is good evidence that the clam mortality was not

caused by high temperatures alone. The summer of 1949 was one of the warmest on record, if we take Boston records as a measure; but 1946, when Galtsoff found rotting clams, was an unusually cool summer. The wide range in per cent mortality on adjoining flats in Plum Island Sound is also evidence against the effect of temperature.

The mortality apparently was not caused by the condition of the soil. Nightingale believed that the Essex mortality of 1914 was caused by decaying plant material, but we found no evidence of this. Rotting clams were found in soils ranging from loose clean sand to firm sandy mud and relatively soft mud. Furthermore, clams in the Merrimack River were doing well in black mud over masses of plant material much like those described by Nightingale. There was no indication of smothering.

Predation by horseshoe crabs, green crabs, boring snails, and birds may be the principal cause of the clam shortage. A predator usually devours its victim and does not waste it by leaving it to rot. However, local diggers report that green crabs nip off siphons and leave clams to die, and this is certainly a possibility. Most of the dead clams found were so far decayed that no injury could be seen, but we have found a few clams with injured siphons.

A few weeks ago we found some interesting variations in mortality of planted clams in experimental plots set out primarily to test methods of predator control. In two plots not protected from green crabs, the mortality was 24 per cent, while there was no mortality in a plot covered by heavy gauge one inch mesh chicken wire only thirty feet away.

It seems hard to believe that the extensive mortality of 1949 and the various cases reported for other years could all be caused by injuries from predators, but it is a possibility worth further investigation.

The other possibility seems to be that some disease is killing the clams. Diseases have been described in oysters and in practically every organism that has been thoroughly investigated, so it would be logical to assume that clams have diseases also. This possibility is being investigated by bacteriological and histological work at Newburyport.

The Hard Clam (Quahaug) Program

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Biological studies on the quahaug were begun in Rhode Island, with preliminary investigations on larvae, growth, and effects of types of gear, in 1949 and have been outlined by Mr. Glud at a previous meeting of this group. Preliminary outlining and planning of a productivity study, similar to that already begun on soft clams, was started in the winter of 1949-50.

The objectives of the program are as follows:

1. To determine the physical and biological conditions necessary to maintain an area at maximum production over a long period or to restore a depleted area.

2. To determine if an understanding of any of these conditions can be utilized to maintain production or overcome depletion.

3. To develop methods which may be used by other conservation agencies to attack problems of quahaugs in their own localities.

Rhode Island was selected as the location for these studies of quahaugs after extensive contact with members of the industry. Two coastal surveys were made from Maine to Florida. Dealers and fishermen were interviewed and problems of the industry discussed. Rhode Island was finally selected as the central area and permanent headquarters for hard clam research were established. Rhode Island offered four inducements which influenced our decision:

1. The area is highly productive and has an intensive quahaug fishery.

2. The productive areas are small enough to be easily and intensively studied and for the most part are sheltered for maximum working efficiency.

3. The Narragansett Marine Laboratory extended the use of research facilities, office and laboratory space and a vessel, and is conducting cooperative studies.

4. The proximity to the other Clam Investigations units at Newburyport, Massachusetts, and Boothbay Harbor, Maine, permits more frequent meetings

of investigation personnel for exchange of ideas and for more efficient administration.

Greenwich Bay was selected as the outdoor laboratory for the productivity studies. The bay is located on the western side of Narragansett Bay about thirteen miles from the ocean. The total area is approximately 2700 acres, and maximum depths range from 10 to 25 feet at flood tide.

For many years Greenwich Bay has been one of the best quahaug-producing areas in the State and supports about 20 full-time bull-rake and tong fishermen. During the summer this number is more than doubled. Eight shellfish dealers are located around the bay, but one dealer handles more than three quarters of the catch.

The first survey of Greenwich Bay was begun June 12; (1) to study the nature of the bottom and (2) the numerical and size distribution of quahaugs. A grid of stations 600 feet apart was laid down over the area. Samples were taken at these stations with a construction type clam-shell bucket. The bottom material was screened and the quahaugs counted and measured. This survey will be repeated again in September, and in each succeeding spring and fall for at least two more years. From these repeated surveys we hope to be able to estimate the rate at which quahaugs are being removed by the fishery and the rate at which they are being replaced by natural spawning. If we are successful in developing our estimate, our next step will be to determine the maximum annual removal coincident with sustained production.

Preliminary analysis of the survey data showed the bay to be divided roughly in half by two types of bottom. The western half is predominantly sticky mud, and the eastern half principally sand, with small areas of shell mixed with sand. Approximately two-thirds of the bay appears to be producing quahaugs. Most of the small (under legal size) quahaugs are concentrated in the western part. "Neck" size clams are found in patches all over the bay, while "large" quahaugs are located predominantly in the eastern part. One of the outstanding preliminary observation of the survey is the irregularity of distribution of the clams. The centers of heavy concentration were small in area and widely scattered over the bay. This confirms evidence we have obtained from interviewing fishermen, and is probably characteristic for most quahaug-producing areas of Narragansett Bay.

Studies were begun in July to determine the possible relation of tidal currents to the distribution, setting and survival of larval quahaug. We were interested in knowing whether the populations in Greenwich Bay were self-sustaining, or if larvae could be brought in from other closely adjacent areas of Narragansett Bay. Sheet-metal crosses were supported at different depths from small cork floats to determine the direction and

rate of flow of the water at different levels. These were followed for entire tidal cycles, and the movements plotted on charts of the area. These experiments are not completed and no results can be reported at this time.

Plankton samples are being taken three times a week at two locations in the bay to study occurrence of larvae. A heavy spawning apparently occurred in late June, but only a small number of larvae have been found since that time.

An interview system has been started on Greenwich Bay to estimate the extent of removal by the commercial fishery. To obtain maximum accuracy, we question the men on the fishing ground as to their catch, hours worked, and any information they may be able to offer on seasonal aspects and trends of the fishery. Experiments are planned for the near future to determine the feasibility of "farming" quahaugs. From these we expect to obtain information on the most favorable type of bottom, best patterns of tide or current, and the effects of crowding on the growth of clams of various sizes.

It is too early in the investigation of quahaugs to have more than preliminary results. Only since May, 1950, have we had the minimum required staff and equipment necessary to conduct our program. At present we can simply outline our operational techniques, and state what information we expect to gain from these studies. This is as follows:

1. An estimate of the expected annual yield at present fishing levels.

2. The best removal for maximum yield over a long period.

3. The conditions of life history and ecology which influence production.

4. Basic knowledge of farming methods.

In addition to the Rhode Island studies, cooperative and supplemental projects are being conducted in other localities. Dr. V. L. Loosanoff, Director of the U. S. Biological Laboratory at Milford, Connecticut, is directing his efforts toward laboratory culture of clams and is assisting in the identification of larval stages of common mollusks by preparing photomicrographs and microscope slides for distribution to workers throughout the investigation. He has successfully reared large numbers of quahaug larvae past setting size, and has supplied them to investigators in several areas for experimental planting.

Dr. Thurlow Nelson of Rutgers University is directing graduate students in a continuation of his 1949 program, devoted to the study of food organisms and the problem of obtaining seed from natural reproduction. Dr. H. Haskin is conducting studies

on the growth of clams of various ages, the seasonal increase in the size of meats, and the effects of predators on seed clams. Dr. M. Carriker is investigating pelagic bivalve larvae to establish criteria to differentiate the larval stages of quahaugs from those of other mollusks.

Dr. Charles J. Fish, Director of the Narragansett Marine Laboratory is conducting an overall ecological survey of Narragansett Bay, placing special emphasis on the quahaug populations.

Dr. Walter A. Chipman, of the Fish and Wildlife Service Laboratory at Beaufort, North Carolina, is conducting a study of the basic food materials of clams, employing newly developed radio-active tracer techniques.

About 1100 hard clams have been planted in Maryland in cooperation with the Maryland Department of Research and Education for growth and survival studies in this area. Another smaller planting in the Beaufort area has been made by Dr. Chestnut of the Institute of Fisheries Research of North Carolina.

Observations on the Life History of the Sea Scallop and Its Fishery in Maine

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The sea scallop, Placopecten magellanicus, is one of two principal commercial scallops found on the Atlantic coast. It may be distinguished from the bay scallop, Pecten irradians, by its larger size, its lack of prominent fluting upon the valves, its possession of separate sexes, and especially by the differences in bathymetric and geographic ranges, since the bay scallop is more restricted to shallow, inshore waters from southern Maine, southward.

This larger mollusk grows to a diameter of about 8 inches, but most marketable catches average 4½ inches. The shell is rounded in outline and strongly flattened, the valve upon which the animal rests being much flatter than the upper one. The valves are rather light, lending themselves to the swimming ability of the mollusk. They do not fit tightly together, and leave the scallop susceptible to starfish attack and prevent shipment of the product in the shell. Fine striations radiate from the umbo to the margin and growth lines are evident, concentric to the margin. Winter checks occur in the growth lines and are indicative of annual growth.

A live scallop, lying relaxed, with its valves slightly open, shows two rows of eyes around the margin of the shell, one row on each mantle edge. Each eye is perfectly formed and constitutes a rare structure among most bivalves, but does not seem to perform any important sensory function of the animal.

One of the most prominent features of the internal anatomy of the scallop is the adductor muscle, stretching from valve to valve. This muscle is the "meat" to the fishermen and the "scallop" to the market. It is made up of two distinct parts, the larger of which is adapted for rapid contractions and aids in swimming, while the other, smaller, portion is adapted to slower and more forceful action, such as holding the valves closed when danger threatens.

The other prominent feature of the body of the scallop is the gonad. The sexes are separate in this mollusk, and at maturity a distinct difference may be seen between male and female. The sperm are white and the ova are brilliant orange to red. The sex ratio is nearly one to one and both sexes mature at the same time.

A scallop begins its life in August or September, when the spawning season occurs. As in the case of many other mollusks, fertilization takes place in the water, when ova and sperm, released from separate individuals, meet and unite. The early stage of the scallop is free-swimming until sufficient size and shell growth are attained to cause it to settle to the bottom. Once settled, it attaches itself to rocks or shells by means of thread-like glandular secretions. It remains so attached for a rather indefinite period, generally over a year.

Feeding upon microscopic plankton forms and living at depths of more or less constantly low temperatures, the mollusk reaches a diameter of 2 3/8 inches during its third summer. A few individuals may reproduce at this time, but most sexual maturing occurs at four years of age, when a diameter of 3½ inches has been reached. Up to this time, the scallop is very active and moves about at the slightest provocation. The swimming action is accomplished by opening and closing the valves in rapid succession. The water, taken in by the opening of the valves, is forced out by their closing, and its direction of flow is controlled by the muscular free edges of the mantle. Older scallops are much less active and accumulate heavy growths of various fouling organisms. Individuals as old as 19 years have been recorded. Undoubtedly there are older ones, but aging is complicated by erosion of the shell, the slowing of growth, and the resulting indistinctness of the winter check rings, by which growth is calculated.

The scallop does not suffer from a great number of enemies, and aside from man, only one is very serious. The starfish common to its geographic and bathymetric range is Asterias vulgaris, which preys heavily upon all sizes of this mollusk. The smaller scallops are easily opened by the larger starfish, and the larger mollusks, lacking a tightly closing shell, are subject to entrance by smaller starfish and to the insertion of the everted stomachs of the larger predators.

The valves of the scallop are eroded by species of the boring sponge genus Cliona and by the boring clam, Saxicava arctica. This action weakens the shell, but does not seriously harm the animal. It is believed that more extensive erosion occurs in this mollusk than in most others because it does not burrow and because it lacks an adequate protective cuticular covering on the shell.

Other questionably adverse conditions affecting the welfare of the scallop are: the handling of undersized individuals on deck, especially in sub-zero weather; the action of the dredge in disturbing the bottom and burying small sizes; and littering the beds with waste portions of the mollusks caused by shucking over the beds.

Several common inhabitants of the shells of live scallops

are such bottom fish as the rock eels, sea snails, and young squirrel hake. As far as is known, these forms do not harm the mollusks in any way.

Sea scallops exist in accumulations generally known as beds, the location of which seems dependent upon the nature of the currents and the topography of the bottom. The beds generally occur in a long, elliptical shape, lying lengthwise parallel to the current and in a bottom area of lower elevation. They are found on all types of bottom, from coarse rocks to mud, but setting of the early stages occurs more favorably on gravelly or rocky bottoms.

The range of this mollusk is from northeastern Canada to just south of Long Island, and its optimum range appears to include the coast of Maine and especially Georges Bank. The bathymetric range is from one to 150 fathoms, while dead shells have been found as deep as 400 fathoms.

The free-swimming ability of the sea scallop has given opportunity for many suppositions concerning its possible migratory habits. Many scallop fishermen tell of mass evacuations of favorite beds, while just as many can tell of the sudden occurrence of beds where none formerly existed. Investigations so far have been able to show only movements from shallow into deeper water.

The sea scallop fishery in Maine is believed to have started in the town of Mt. Desert on Mt. Desert Island in 1884. The principal dredging grounds were soon extended to include the area between the Sheepscot River and Mt. Desert Island. The dredging gear in use at the time was very light, consisting generally of oyster dredges or various modifications, attached by rope and moved by either hand or sail, so the more shallow beds, down to six or eight fathoms, were preferred.

The scalloping season at this early period depended chiefly upon the proximity of the market. When there was good local demand, the fishing continued all year. When only the distant markets were available, the season depended upon the cold weather which furnished protection for the shipments. The principal market at this time was Boston, with smaller amounts going to Maine cities and to New York and Philadelphia.

During this period, the existence of beds in deeper water was known, and the need for improved gear for fishing them was recognized. The United States Fish Commission had demonstrated that a beam trawl was the most successful type of gear, except that smooth bottom was required to prevent tearing the net.

The present fishery takes place during an open season from November through March and furnishes valuable supplementary income for the many lobster fishermen who do not continue their work through the winter months. Maine has scallop beds along

the major part of its coastline, but those of principal production occur from Penobscot Bay eastward. Considerable advances have been made in the type of gear and methods used. The majority of vessels are in the 30- to 40-foot class and have been converted from lobstering to scalloping by the installation of a winch, mast, and hoisting gear.

Much heavier dredges are now in use. One of the two principal types employed has a mouth six feet wide, preferred for muddy bottom, and the other is made up of two dredges, each with a three-foot mouth, attached side by side to the same yoke, and preferred for rocky bottom. The bag of a dredge has an iron ring mesh on the bottom, and a twine mesh above.

Towing is done by means of wire cable running to the winch on deck. By means of this gear, beds down to 50 fathoms are easily fished. Tows of up to a mile are made, depending upon the character of the bottom and the abundance of scallops. The dredge is hauled up, its load dumped on deck, sorted, and the scallop meats shucked out while the next tow is being made. Most trips are of only one day's duration.

In port, the fresh product is sold, either to local retailers, or to wholesalers. In 1948, a little over 450,000 pounds of meats were landed in Maine, and sold at about $0.48 per pound. In 1949, over 500,000 pounds were landed, and sold at about $0.35 per pound. It may be seen that, far from being merely a business, supplemental to summer lobstering, the Maine scallop fishery is one of the state's important sources of income for fishermen.

Report on Various Tests on Bottoms for Oyster Planting

By

William H. Dumont 1/

At last year's convention, Mr. Allen Sollers of Maryland spoke on testing of potential oyster bottoms or grounds by the use of a sounding pole. The success of this method is based on the experience and judgement of the person with the pole. If several bottoms were tested by three or more people, there would be general agreement on many of the types of grounds tested, but on some, there would be wide disagreement.

The old Bureau of Fisheries decided in 1929 to try and work out laboratory methods whereby bottoms suitable for oyster culture could be distinguished from those which are not. At that time there was no one test or tests which could be made quantitatively and on an unbiased basis. It was hoped that such tests could be found or devised. The tests I will describe were carried out in 1930 and 1931 in one of the old Bureau of Fisheries' laboratories. If a large number of samples are to be examined, the tests should be performed in a minimum of time and with as simple equipment as possible.

TYPES OF OYSTER BOTTOMS: What are the types of bottoms used for oyster culture? Our sampling has shown that in Delaware Bay, the inshore grounds are sandy muds while the off-shore grounds are mostly sands. Egg Island Bar is not planted now, because the sand on the top of this bar will shift during a storm.

In Maryland, we found that sands, hard and semi-soft muds were in use for the cultivation of oysters. In Virginia, hard clay, sand and soft bottoms are being utilized. In some parts of the York River and on the south side of the James River, the bottoms are so soft that it is necessary to plant 800 to 1200 bushels of oysters per acre, for if a lesser amount were used, the oysters would sink and be lost. Oysters can be taken only by tongs, since dredges would break through the thin upper layer. Local oystermen often first plant shells to stiffen the top before seed oysters were planted. Yet nearby there are other bottoms which, from experience, the oystermen have found could not be used for oyster planting, although treated in the same way.

In Mobjack Bay, the upper part of the planted area is soft mud, while the lower part is a sandy mud.

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In Georgia and parts of South Carolina, below the low water mark and on what is known as the "ebb tide" side of the creeks and streams, the bottoms are hard clay and sands. Between tide marks and on the "flood tide" side of these creeks, the bottoms are of the soft and very soft mud types. Oyster planted on the latter type will disappear over night. Only clumps of coon oysters are found there.

I have not sampled the oyster grounds of Long Island, Connecticut, or Rhode Island, so can not include their types.

Dr. Paul Galtsoff collected the first series of 47 samples in 1925 from bottoms in Georgia while making a survey of the possibilities for utilizing certain creeks and streams for oyster culture. These were classified, from the field notes, as (1) suitable for oyster culture--hard muds and sticky muds, (2) probably suitable--sand and mud, and sand and sticky muds, and (3) unsuitable--sand, shifting sand, soft mud and very soft mud. These bottoms were mostly unproductive.

A preliminary mechanical analysis by the beaker method of Thoulet (1) was made on 14 of these Georgia samples in 1929 by H. F. Prytherch. These seemed to show some relation between the amount of clay (particles less than .005 mm. in diameter) and the hardness of the bottom.

In the beaker method by Thoulet, no oxidizing agent is used to remove the organic matter and no dispersing agent to aid in the separation of the silt and clay from the sand. Mechanical analysis is supposed to separate the various grades of sand, from the silt and clay. Mechanical analysis by Thoulet's method was made in 1930 by the writer on all 47 Georgia samples. In this first series, the mechanical analysis of these Georgia bottoms did not show any correlation between the amount of clay present and the suitability of the bottom for oyster culture.

A more detailed mechanical analysis was made in the U. S. Bureau of Soils laboratory on 8 of these same samples, using the pipette method and a dispersing agent. Three of the samples were from the hard mud and one of sand and mud, from the suitable bottoms, and 4 from the soft mud or unsuitable bottoms. The results showed that in six of the 8 samples, the beaker method gave a lower clay content than by the pipette method. It also showed that a dispersion agent was necessary to obtain accurately the amount of silt, clay and colloidal clay.

But all these samples in the first series had been air dried for 5 or more years and were from unproductive bottoms in Georgia. To check this further, a second series of 28 bottom samples was collected from known oyster producing grounds and from adjacent barren bottoms. These were taken in New Jersey, Maryland, Virginia and Georgia in 1930 and 1931. These were grouped as (1) oyster producing grounds--hard mud, sand, and soft mud, and (2) non-producing bottoms--shifting sand, soft mud and very soft mud.

Of the oyster producing grounds, the hard mud type was represented by only one sample from ebb-tide side of Jointer Creek, near where it joins Deep Creek. This is near Brunswick, Georgia. Sandy samples were collected as follows: 2 from the Patuxent River in Maryland; 2 from Delaware Bay in Outer Deep water and near Egg Island Bar; and 1 from south side of York River, Virginia. Sandy mud samples: 2 in Patuxent River, Md.; 1 from Piankatank River, Va.,; and 2 from Miles' grounds in Lower Mobjack Bay, Va.; and 1 from Inner Deep Water in Delaware Bay, N.J. Soft mud bottom samples were from: 1 from Solomon's Island Harbor, Md.; 1 each from Miles and Darlings' grounds in upper Mobjack Bay; 1 from Piankatank River; 1 south side of James River on Nansemond Ridge, Va.; and 1 from a ground off Kenney's Point in Maurice River Cove section of Delaware Bay, N. J.

Samples of the non-productive or barren bottoms were: Shifting sand type: 1 sample each from Egg Island Bar, Delaware Bay; the bar at mouth of Patuxent River, Md.; and from Jointer Creek, Georgia. Sandy mud, 1 from upper Mobjack Bay, Va. Soft mud type: 1 from Back Creek, near Solomon's Island, and 1 from Solomon's Island Harbor. Four samples of the very soft bottoms: 1 each from the channel into Maurice River, N. J.; the channel of North River, Va.; off Barrel Point on south side of James River, Virginia; and on flood tide side of Jointer Creek, Georgia.

The mechanical analysis, using the pipette method and on undried samples, was made on these 28 samples. The results confirmed, with two exceptions, those obtained from the first series, that is: there was no correlation between the clay content and the suitability of the bottom for growing oysters. The exceptions were the hard mud from the ebb tide side of Jointer Creek, Georgia, where oysters were growing, and the very soft mud from the flood tide side of the same creek and where no oysters were growing. Both samples were taken with 50 feet of each other. Mechanical analysis showed that there was very little difference in the amount of sand or in the colloidal clay. We had this checked by the U. S. Bureau of Soils which ran additional tests. The only difference it could find between these two samples was in the amount of organic matter. It stated that both samples were of the very soft muck type and unstable. We had the U. S. Bureau of Public Roads test these two samples. It reported that both samples represented colloidal clay soils of very unstable variety. This lack of stability, as shown by the shrinkage test, was "caused by the presence of some highly porous material, possibly organic matter."

Next we took these two Georgia samples to the petrological division of the U. S. Geological Survey. It found that the part designated as clay in both samples was composed mainly of diatoms and with a size several times greater than true clay particles.

But the estimate of clay by mechanical analysis is based

on the assumption that the settling particles are spherical. The examination of the Geological Survey showed that, for these two samples, this was not true and that the percentage of clay was too high.

The tests of both the Bureau of Soils and the Bureau of Public Roads were made on air dried samples, using distilled water. No consideration was given to the various salts present and their possible effect on the physical properties of the bottoms. The organic matter may act as a protective coating on the clay particles, and thus prevent them from coagulating.

In general, mechanical analysis did not tell the difference between suitable and unsuitable bottoms for oyster culture.

All the tests on these two Georgia samples classified them as being alike. But one was a hard clay or mud and the other was very soft mud. The difference must be in the colloidal clay particles which comprised over 60 per cent of the entire sample. In the very soft mud type, each clay particle was separate and seemed to have no affinity for each other, while in the hard clay sample the colliod clay particles stuck together and coagulated or acted as a binder to the silt and sand. We have something like the same thing in agricultural soils. Some will erode easily while other soils are non-erosive. The Bureau of Soils has worked for many years on why there is this difference between certain soils. Middleton (2) and his co-workers have found that, of the chemical and physical properties, those having the greatest influence on soil erosion, were: the dispersion ratio, the erosion ratio, the silica sesquioxide ratio and the ratio of colloid clay to the centrifuge moisture equivalent. Non-erosive agricultural soils have a dispersion ratio of 15 or less while soils which erode easily have a higher ratio. As well as mentioned later, the same ratio seems to hold true for oyster bottoms.

In our discussions with various Federal agencies which conducted the different tests on these two Georgia samples, we learned of the tests worked out by the Bureau of Public Roads for the testing of subsoils in roadbuilding.

It also found that mechanical analysis in itself told nothing about the physical properties of the soils. It worked out a large number of tests which have since been reduced to seven simple ones, which, taken together, show what may be expected of a soil for road construction. These tests can be made in a much shorter time than the mechanical analysis of the Bureau of Soils or that used in our laboratory. These seven tests are: mechanical analysis by the hydrometer method, lower liquid limit, plasticity index, shrinkage limit, shrinkage ratio, centrifuge moisture equivalent and field moisture equivalent. From results obtained in the field and with these tests, the Bureau of Public Roads, (now known as the Public Roads Administration)

has worked out a classification of soils for road building. We requested the Bureau of Public Roads to try its subsoil tests on these two Georgia samples. These tests immediately showed a large difference between the hard clay productive bottom and the very soft unproductive bottom. As a further check, 21 other samples from the second series were tested by the Bureau of Roads and also in our laboratory.

These tests for subsoils are relative easy, can be made within a comparatively short time and do not require a large or elaborate amount of equipment. I will not describe or discuss these tests, since anyone interested can obtain the procedures from the June and July 1931 issues and the February 1942 issue of Public Roads, published by the Public Roads Administration, Washington, D. C.

The hard clay bottom from Georgia had a dispersing ration of 8.8 while all the other samples were above 37. The erosion ration for this hard clay was 18.8, with all the others above 50. This is what would be expected and agrees with what Middleton (2) of the Bureau of Soils has classified as a non-erosive agriculture soil. The dispersion ratio distinguishes between the hard muds and the other types of bottoms. The plasticity index spotted the sands and the sandy muds. None of these tests seem to make any differentiation between the productive sands and sandy muds, and the unproductive shifting sand or sandy mud bottoms. The dispersing ratio, the lower liquid limit, plasticity index and field moisture equivalent seems to separate the soft productive oyster bottoms from the soft unproductive bottoms. The erosion ratio, centrifuge moisture equivalent and field moisture equivalent may be useful to tell the very soft unproductive bottoms from the soft bottoms. But the chief value of the constants obtained in these tests seems to be in the relations existing between some of them rather than in the magnitude of the individual constants considered separately.

The relation between the lower liquid limit and four of the other test constants (plasticity index, shrinkage limit, centrifuge moisture equivalent and field moisture equivalent) show these differences more clearly.

But not enough samples of hard clay or mud bottoms have been tested to make a definite statement that these differences will be found for all types of hard oyster bottoms. This work had to be discontinued in 1931 as the writer was transferred to other work in another Section of the Service. As to the other types of bottoms, the results on the 23 samples seem to indicate that many of these subsoil tests may be useful in classifying marine bottoms into definite groups.

A table has been prepared showing the range of constants as found by these various subsoil tests for these 23 samples.

Based on these constants, a tentative classification has been proposed.

1. (a) Dispersion ratio below 15 ..... hard muds

   (b) Dispersion ratio over 15 ..... see 2

2. (a) Field moisture equivalent below 30, and plasticity index below 10 ..... see 3

   (b) Field moisture equivalent over 30, and plasticity index over 21 ..... see 4

3. (a) Shrinkage limit above 15 ..... sandy muds

   (b) Shrinkage limit below 15 (generally below 10).sands

4. (a) Dispersion ratio 50-77; plasticity index 20-26; field moisture equivalent 30-43 ..... soft productive muds

   (b) Dispersion ratio 77-85; plasticity index 26-32; field moisture equivalent 44-50 ..... soft non-productive muds

   (c) Dispersion ratio 35-60; field moisture equivalent 50 and up; centrifuge moisture equivalent 63 and up ... . very soft non-productive muds

SUMMARY: Mechanical analysis by the two methods used is not an aid in distinguishing the type of bottoms suitable for oyster culture. There seems to be no correlation between the character and size of particles and the consistency of the bottom.

The tests devised by the Public Roads Administration for subsoils seems to be the most promising ones available at present for telling the various types of bottoms. With more samples from the different types of oyster grounds in all sections of the country, it may be possible to work out an accurate basis for analysing them in the laboratory on a scientific scale.

It is known that other oyster research investigators have considered various methods and tests for oyster bottoms. It is hoped that the tests described in this paper may be of assistance to those who may have an opportunity to carry further this line of research.

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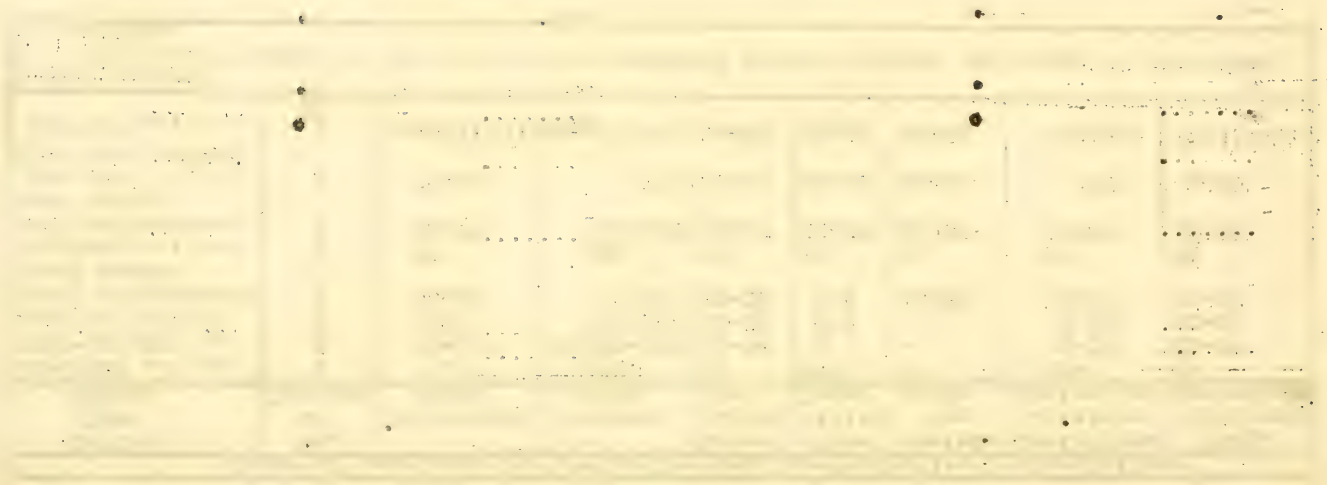
Range of Constants for Productive and Non-Productive Oyster Bottoms

Bottom Type Bottom	No. of Samples	Dispersion Ratio	Erosion Ratio	Lower Liquid Limit	Plasticity Index	Shrinkage Limit	Centrifuge Moisture Equivalent	Field Moisture Equivalent
Hard mud	1	8.8	18.8	94	47	27	129*	69
Sand-oysters ...	5	90-100	(87)33-333	19-24	0	-	4-19	21-28
Sand-no oysters	2	100	195-343	19-25	0-5	-	4-23	23-25
Sandy mud-oysters	3	53-69	79-83	22-25	6-8	19-23	16-27	21-27
Sandy mud-no oysters	1	67	100	24	10	19	17	21
Soft mud-oysters	5	50-77	101-146	42-58	22-26	22-30-	42-55	31-43
Soft mud-no oysters	2	77-85	-176	56-61	26-32	23-33	-81*	44-49
Very soft mud-no oysters	4	37-54(100)	46-82(145)	54-96	28-45	20-36	63-121*	(37)57-61
	23							

*water logged
( ) indicates 1 sample of the group had a constant widely outside the range of the other samples.



A Brief Report on the Texas Oyster Investigation

by

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Natural production of oysters in the bays of Texas has become a thing of the past in view of the tremendous decline from 200,000 barrels produced in 1904 to about 7,000 barrels produced this past year. Overfishing and neglect of the reefs is probably the principle factor for this decline and the establishment of systems of commercial cultivation appears to be the main solution to the problem.

The sheltered bays along the Texas coast provide a suitable habitat for oyster cultivation and though the environment is in many respects dissimilar to that of the Eastern Seaboard intelligently conducted cultivation should produce marketable harvests in quantity.

Since 1947 members of the staff of the Marine Laboratory, Texas Game, Fish and Oyster Commission, at Rockport have been engaged in a research program designed to secure data on growth, mortality, spawning and spatfall, enemies, and the physical environment of oysters as an aid to the determination of suitable culture methods.

As a preliminary step in this investigation, six one acre plots containing varying amounts of transplanted seed oysters were established. These plots were located to include as many variations in bottom and hydrographic conditions as possible. Periodically growth measurements, mortality rates, salinity, temperature, and spawning observations were made. Results on the whole were not favorable although in several areas the locations chosen were for the purpose of determining the extent of utilization of poor quality bottom. Mortality was generally high, raging around forty per cent and in one location several plots were decimated by the oyster drill (Thais floridana floridana). Average growth over about a six month period amounted to approximately one-half inch increase.

In 1948 two types of cultch were placed at one location, these being dry shell spread evenly over the bottom and bundles of bay brush anchored to concrete blocks. By August of that year the shell showed a catch of over two spat per shell and one year later an average catch of over one spat per shell. Due to rapid fouling by algal growth, the brush collected practically no set, however, it is believed that under more suitable conditions a satisfactory strike could be obtained on this type of cultch. There was little spat survival because of drill depredation.

During the season of 1948-1949 three additional seed plots were established using specially constructed dredging equipment which materially reduced the cost of this operation. Mortality was somewhat less than the pre vious year, averaging around thirty per cent and the growth rate remained at about the same average. Approximately 7,000 adult oysters were placed on a sea rack in November, 1948; examination the following year showed an excessive mortality by drills and probable trespassing.

Shell bag spat collectors were placed in an area of heavy spat fall during August, 1949, with counts being made every few days until early October. The average set per shell ranged as high as sixteen spat. Similar collectors are being used this season but examined on a monthly basis, the initial set averaged around eight per shell and at the end of three months had increased to about twenty spat, measuring up to one inch in length. Plankton analyses have been made regularly throughout the area with particular reference to the abundance and distribution of oyster larvae. This year emphasis is being placed upon this phase of the investigation. Straight hinge larvae have been observed as early as March and there is the possibility that spawning may continue into November. In all bays under study except one, the spatfall appears to be adequate if not excessive although mortality rates are often high, probably due, in some instances, to the relatively high summer water temperature in shallow depths.

During January of this year a survey was made, with the greatly appreciated assistance of Dr. Philip A. Butler of the U. S. Fish and Wildlife Service, of a number of reefs in eight of the bay areas. This survey showed strongly the deplorable conditions existing on many of our natural reefs as relatively few indicated capabilities of producing market oysters. After consulting with Dr. Butler, a series of recommendations were considered for the long-term objectives of this research program. The objectives adopted are as follows:

1. Further study of the environmental features is necessary to determine areas suitable for the cultivation of marketable oysters, together with investigation of the costs and methods for improving and expanding natural reefs as well as for establishing new reefs. Data on the physical and biological factors pertaining to oyster quality and productivity should be obtained.

2. It will be necessary to evaluate properly the existing and potential oyster resources. Surveys should be made of the size and location of existing reefs with analyses of their population.

3. Factors affecting oyster culture should be studied where localized problems in pollution may occur.

4. Investigation of the fundamental aspects of oyster biology and the devising of experimental techniques to verify or evaluate field observations are necessary parts of this investigation.

5. Dissemination of scientific data so collected should be made available to the public and to legislative bodies for their information in enacting conservation laws relating to the oyster.

Naturally the mechanics of conducting this investigation are large in scope, however, proper collection and evaluation of data should result in the achievement of a better understanding of the problems in oyster production on the Texas coast.

Recent Observations on the Season and Pattern of Oyster Setting in the Middle Chesapeake Area

by

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In order to carry out effectively an extensive shell planting program much detailed information on the normal distribution and time of spat fall is needed. Maryland inaugurated a state shell planting program when few data of this nature were available for local bars. No records were kept of the set received by most of the earlier plantings and only during the past ten years has an attempt been made to examine and record systematically the effective set found upon all state shell plantings at the end of each spawning season. Counts of the spat received by natural cultch on representative bars throughout the State also have been made on an increasingly widespread basis during recent years. Accumulation of such data was begun at Solomons and has now been expanded as part of an extensive program participated in jointly by personnel of this Laboratory, the Department of Tidewater Fisheries, the Fish and Wildlife Service and the Virginia Fisheries Laboratory.

The time of oyster setting can readily be determined by the usual method of exposing spat collecters at intervals during the spawning season and examining them microscopically. A number of excellent studies of this nature in the Chesapeake area have been made and published. Spat collecters also are useful in indicating the potential set which might be obtained at various locations through properly timed shell plantings. Accumulated data have shown that, upon the many diverse bars of the Chesapeake area, the season and pattern of setting may vary markedly from place to place and from year to year. It is likely that changes in the amount and location of brood stock which result from harvesting operations may further change the trend of setting. Continued and extensive observations of setting are needed in order that the most effective use may be made of planted cultch in this region where production of sufficient seed oysters is a major problem. Studies of the many factors which may control the wide variations in setting are being made by numerous workers in this and other areas. Comparison of setting records affords one of the means by which factors which influence general setting trends may be found.

The year 1949 may be classed as one of somewhat better than average set over much of the Chesapeake oyster area in Maryland. The accompanying chart (Fig. 1) shows the average spat count per Maryland bushel for different regions of the State. Most of these counts were made during the late fall and winter months

when the spat were generally around an inch in length. In a few instances the figures may represent only one or two bars but most of them are averages for a number of bars for which details cannot be shown on a chart of this size. It will be noted that setting was very poor along the upper Western Shore of the Bay and in the major rivers except near their mouths. The tendency towards higher setting along the eastern side of the Bay agrees with observations of previous years. It is in accord with the findings of Dr. Nelson in Delaware Bay where he attributes the distribution of oyster larvae to the diversion, in the northern hemisphere, of the entering wedge of salt water along the bottom of estuaries towards the right bank as the denser water moves upstream. This diversion is a physical result of the spinning of the earth. The Potomac River and a few of the larger tributaries show a similar trend in setting.

Newly planted shells usually receive a heavier set than do old shells which have become weathered and heavily coated with fouling organisms. Certain areas in Maryland whose past record indicates satisfactory setting and which possess sufficient acreages of suitable bottom have been designated as seed areas. Large plantings of shell are made annually by the State in these areas later to be transplanted to good growing bottom after receiving a satisfactory act. Other bottoms of smaller acreage where moderately good sets may be expected are shelled lightly and the resulting catch left to mature without transplanting. Figure 2 shows the set received in 1949 on all shell plantings of that year with the four areas designated for seed production so marked. It will be noted that the seed area in Eastern Bay failed to receive sufficient set to be utilized as seed. Also certain plantings not intended for seed production received too many spat to mature into the most desirable type of market oyster unless thinned by transplanting operations. In general the spat counts on planted shell were considerably higher than those made on old cultch in the same areas.

Periodic exposures of experimental cultch, mostly in the form of clean oyster shells in duplicate wire bags, have been made in various parts of the Chesapeake area by the several research agencies. Special emphasis has been placed upon seed areas in order to indicate how better timing and placement of shells might produce higher sets.

Figure 3 shows graphically the time and intensity of 1949 oyster setting upon test shells at four Maryland localities. All shells were reasonably clean ones selected from a commercial shell pile but were not specially washed. They were exposed in random positions in small chicken wire bags. Ten random inner shell faces from each bag were examined under low power binoculars after exposure. The area of each shell face is recorded and the results have been expressed as spat per day per shell inner face of a standard area of 50 $cm.^2$ Parker Moore is a typical bar in the Chesapeake just above the mouth of the

Patuxent River. It is a dredging bar and is still in production. The Swash is a productive tonging bar just inside the mouth of the Patuxent River. Both of these have a relatively poor setting record although the set of 1949 is somewhat above the recent average. Holland Straits is an extensive shallow water area lying between low marshy islands which separate the Chesapeake Bay from Tangier Sound. It is about 15 miles above the Virginia line and is being used as one of the state seed areas. Seminary Bar lies in the St. Mary's River, a tributary near the mouth of the Potomac, and has produced the best sets during recent years of any of the state shell plantings. The intensity and time of setting in these four areas illustrate typical variations which may be expected among the various bars in the Chesapeake area.

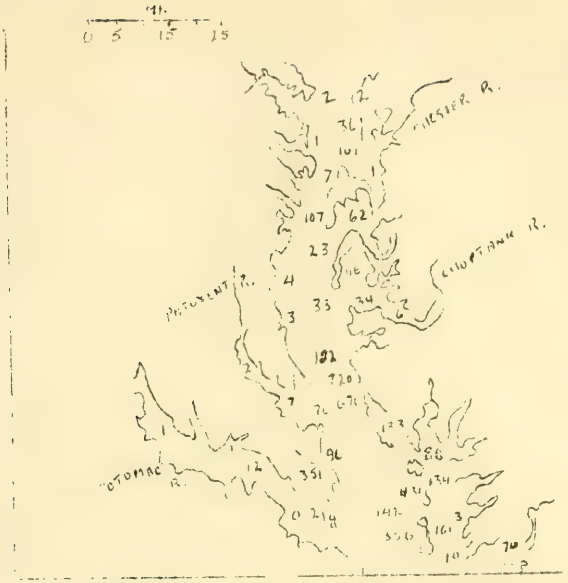
Seminary Bar has been planted repeatedly with shells for seed purposes during recent years. Fig. 4 shows the setting for the past five years on shells exposed in test bags. The commercial set on the state shell planting is indicated at the right. The season of setting on this bar has been rather consistent from year to year although the amount of set has varied. As information on the time of setting of spat and of certain fouling organisms has become available, the time of shell planting has gradually been shifted until most shells have been planted just prior to the beginning of heavy spat-fall during the past two years. There is indication that this policy has resulted in a more effective utilization of the potential set shown by test shells. Extremely heavy initial setting, however, will not necessarily produce a higher commercial set due to mortality caused by overcrowding.

The entire St. Mary's River comprises one of the highest setting areas in Maryland. Fig. 5 shows the 1950 set on 3 bars where shells have been planted at various times for seed production. The intensity of setting this year increased towards the upper portion of the River. Counts of set on scattered shell plantings of previous years show the same trend of increasing set upstream. Data showing a more detailed distribution of set over this area and over the Holland Straits seed area have been gathered.

Qualitative surface plankton samples have been taken in those area where shell bags were exposed. They have shown an abundance of oyster larvae prior to and during the period when heavy setting occurs. Areas receiving little spat fall have shown few larvae present in the plankton. From a number of years of observation, it seems characteristic in the Solomons area that extremely light setting tends to be scattered over a long period often extending from early June into October. In the high setting St. Mary's River seed area spawning and setting are peaked into a two to three week period. In the first instance above, many oysters retain their spawn throughout the season and into fall or sometimes into early winter. Oysters in all

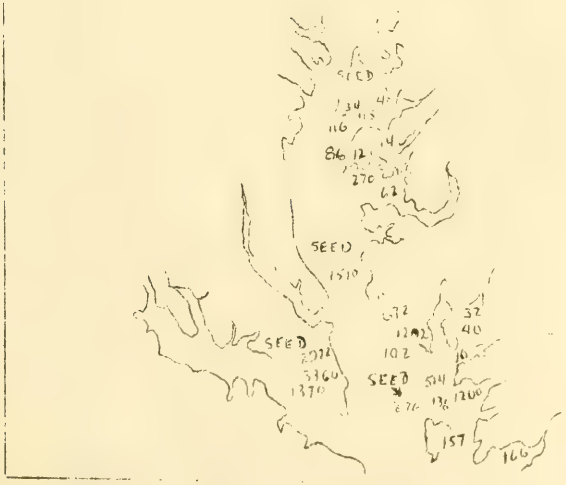
stages of spawning may be found during the summer. In the latter case, practically all oysters seem to be almost completely spawned out after the setting peak has passed and remain thin until cold weather.

The areas of high setting typically are rather landlocked and with less exchange of large water masses than are the portions of the open bay and large rivers where sets are usually poor. Brood stock characteristically is in more densely populated groups and probably is more abundant in proportion to the water volume present. Such conditions may influence the trend towards higher setting. Until more knowledge is gained of the factors controlling setting, a more complete utilization of bottoms whose record indicates favorable setting conditions coupled with retention of well populated areas of brood stock in them seems to offer the best means of increasing production of seed oysters in Maryland.



SPAT/BU OLD CUTCH, AVERAGES, FALL 1949

FIG. 1



SPAT/BU. (FAIL) STATE PLANTS, 1949 SET

FIG. 2

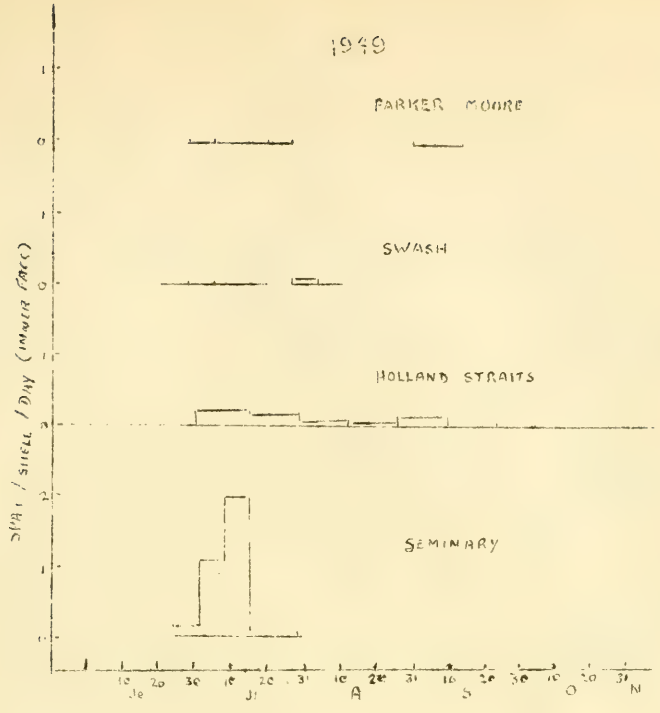

1949
PARKER MOORE
SWASH
HOLLAND STRAITS
SEMINARY
SPAT / SHELL / DAY (INNER FACE)
Je
Jl
A
S
O
N

FIG. 3

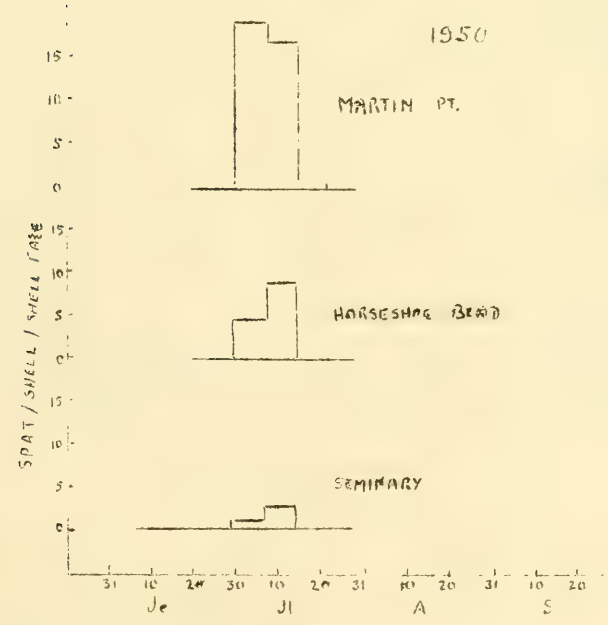

1950
MARTIN PT.
HORSESHOE BEND
SEMINARY
SPAT / SHELL / SHELL FACE
Je
Jl
A
S

FIG. 5

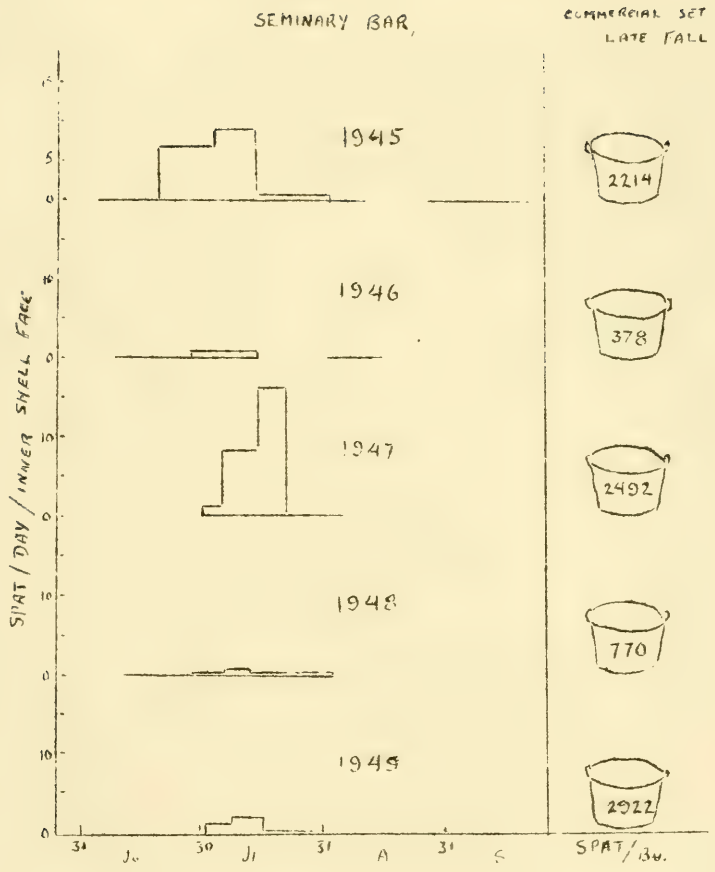

SEMINARY BAR,
COMMERCIAL SET
LATE FALL
1945
1946
1947
1948
1949
2214
378
2492
770
2922
SPAT / DAY / INNER SHELL FACE
SPAT / Bu.
30
Jn
30
Jl
31
A
31
S

FIG. 4

Influence of Seasoning and Position of Oyster Shells on Oyster Setting

by

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The settlement of oyster larvae, which is a critical phase in their life history, has been studied here and abroad for many years with somewhat conflicting results. Nelson in 1926 found in studying the spat fall of Ostrea virginica a ratio of 10:1 in favor of the underside of cultch. Hopkins (1935, 1937) found a ratio of 300:1 in favor of the underside of glass plates when working with O. lurida. Bonnot (1937) found that O. lurida showed no preference between top and underside of collectors used. However, he qualifies this by saying that this may have been caused by turbulence created by the type of collectors used. Prytherch (1928) said that O. virginica set heaviest near the bottom and on the lee side of his collectors. Korringa (1940) observing O. edulis under field conditions found that spat settled heaviest on the upper surface of his test plates. Cole and Knight Jones (1940) found similar results with glass plates immersed in test tanks where O. edulis brood stock was kept. However, theyfound in 1949 that O. edulis larvae set more heavily on the underside of cultch and that this was due to their habit of swimming upward and settling on the underside of any object under which they were trapped. They felt however, that their results, although in favor of setting of spat on the underside of cultch, were not completely conclusive and that other factors strongly influenced the setting habits of O. edulis.

As regards the seasoning of shells, Cole and Knight Jones found that larvae set more heavily on shells which had remained uncleaned for a period of two or more weeks, and which had a film of bacteria and diatoms, than on shells which were cleaned frequently. Those shells which were heavily fouled with sessile organisms were greatly preferred by the oyster larvae over clean shells. This data is quite conclusive regarding the preference of O. edulis larvae. They found that silt was the critical factor in assessing the suitability of cultch. Zobell and Allen (1935) suggested sessile organisms prefer to attach themselves to surfaces covered by a bacterial film rather than a sterile surface. Coe and Allen (1937) found that larvae of O. lurida preferred glass plates that has been used to those which were freshly cleaned. Scheer (1945) also suggests that the presence of a biological film favors the attachment of certain sessile forms. Undoubtedly many ecological factors affect these results and give different answers in different geographical locations. There has been no mention of any work done in the middle Chesapeake area in any of the literature and so it seemed advisable to make an investigation in that area.

These conflicting findings and the lack of evidence concerning the larval behavior of O. virginica led to the simple experiments which are now described. The first investigation was to test the efficiency of shells which were seasoned in sea water for varying periods of time before being exposed to numbers of larvae of O. virginica. Clean shells were immersed in the Patuxent River where almost no natural set occurs for periods of 58 days, 23 days, and 9 days before being exposed in the St. Marys River where large numbers of larvae were known to be present. These exposed shells were carried over to the St. Marys River while immersed in large cans of water and placed in the seed area. At the same time clean shells were placed at random in 1½ inch mesh wire bags and suspended just above the bottom in water of four to five feet in depth. These shells were exposed for seven days at the height of the setting from July 7 until July 14 (Beaven 1950) in the seed area. Unfortunately, the shells exposed for 23 days were lost as a result of tampering. However, the other bags were recovered and ten shells removed from each.

These shells were examined carefully under a microscope and all spat, barnacles, and bryozoa counted. Duplicate bags of shells were examined for each length of exposure so that twenty shells were examined for each length of time they were exposed. Each shell was measured as it was examined and the area in square centimeters calculated. In the results a standard unit of spat per 500 sq. cm. is used in comparing the numbers of spat setting on the different groups of shells.

The results obtained are not conclusive but are suggestive. The work should be repeated and expanded another year as the abrupt ending of the setting precluded the continuing of the work this season.

Table I

Time of Exposure in Patuxent River	Spat per 500 sq. cm.	Remarks
57 days	140	Heavily fouled with Barnacles, Bryozoa
9 days	110	Lightly fouled with Barnacles, Bryozoa
Clean shells	165	Clean shells

The data show no significant differences between the heavily fouled shells and the clean shells and so suggest that more work should be done along this line. Insufficient as the data are, they throw some doubt on the value of clean cultch in obtaining a good set if the cost of getting the shells down just before the set is much greater than planting the shells at a more

convenient and earlier date. In some areas there may be considerable difference in labor costs at different times of the year.

The second experiment was to determine whether larvae preferred the upper or under surface of cultch which was placed horizontally in the water. To test this preference shells were placed, after being scrubbed carefully, in specially made wire mesh bags in which each had a flat piece of asphalt shingle fitted. The scrubbed shells were placed carefully in the bags which were fitted with a harness to keep them flat as they were lowered into the water so that the shingles would lie under the shells and on the bottom. These bags were exposed in the St. Marys River for the same seven day period as those described in the first experiment.

In two of the wire bags the shells were placed cup side up, in two other bags the shells were placed cup side down. After exposure these shells were carefully examined and all oyster spat, barnacles, and bryozoa counted on both sides of the shells, Each bag contained ten selected shells of uniform size and their area in sq. cm. was determined. These shells were exposed at the height of the setting season for seven days in 4-5 feet of water on a natural oyster bar.

The results of this experiment are given in tabular form and are converted to the standard unit of spat per 500 sq. cm. From this data it can be seen that the larvae showed a clear cut preference for the underside of cultch. The upper side of the shells did not show any noticeable silt which would have hindered the setting of the larvae.

The difference in the number of larvae setting on the backs and the faces when both were the undersides may possibly be attributed to the backs actually having a larger area than the faces of the same shells, this difference being caused by the irregularities of the surface of the back of the shell.

It was noted also that barnacles set in almost the same proportion as the spat and showed the same preference for the underside of cultch. Bryozoa seemed to show no preference in their setting habit.

TABLE II

Shells placed face up	Spat per 500 sq. cm.	Barnacles per sq. cm.	Bryozoa per sq. cm.
Faces	90	3.5	30
Backs	530		
Shells placed face down			
Faces	280	25	29
Backs	85		

The preference shown by larvae of O. virginica toward settlement on the under side of cultch agrees with findings of Nelson (1926) except in somewhat lower ratio. The average set on the underside was 405 per 500 sq. cm. and the average on the upper side was 87.5 spat. This agrees with Nelson except the ratio is about 5:1 instead of 10:1. in favor of the underside. This also agrees with the finding of Knight Jones and Cole.

There is an interesting comparison between the shells which were scrubbed and placed carefully in the bags and those shells which were not scrubbed and placed at random in the bags. This showed the scrubbed shells receiving, when both upper and under sides were averaged, a set of almost twice as many spat per 500 sq. cm. as the unscrubbed shells received.

This data was intended as a preliminary study and it is hoped that the work may be continued another year and more conclusive evidence gathered.

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The Selection of Food by the Common Oyster Drill, Urosalpinx Cinerea, Say

Harold H. Haskin

This paper is a report on experiments performed in the summers of 1935 and 1936. It is presented at this late date because the results have not yet been published, and it is believed that they are of some value in providing leads to further work to be done on oyster drill control.

It is common knowledge among oyster growers that the smaller oysters are preferentially attacked by Urosalpinx cinerea, Say, the common oyster drill in New Jersey, In 1910, Dr. Pope, working for the U. S. Bureau of Fisheries, investigated this situation. His results, contained in an unpublished manuscript, were summarized by Professor Moore, also of the Bureau, a year or so later in the statement, "Thin-shelled forms of shellfish were invariably preferred to the thicker-shelled forms." This implies that the drill has an infallible mechanism for finding the easiest way to its shellfish prey.

This idea persisted though Federighi, in a general study of the habits of Urosalpinx in 1931, noted that it would pass by the meats of opened oysters to drill intact oysters in the same dish. Also Professor T. C. Nelson observed in the early 1920's that when drills were allowed to select food from a population of various sizes, there were always smaller oysters among the survivors than some of those that had been drilled.

This observation was repeated in more extensive destruction studies made during the summer of 1935. In these studies, oysters of various sizes, though of the same age, were placed in cages with oyster drills. The drilling in the experimental cages was at random with no apparent size preference shown. The fact remained that in commercial beds the smaller oysters suffered the heavier mortalities. If shell thickness were ruled out as the selective factor, how did the drills choose their victims?

In the destruction studies all the oysters in a single cage with drills had been of the same age, i. e. spawned in the same season, although of greatly varying sizes. In contrast, on the oyster beds, the smaller oysters were, in general, the younger ones. This suggested that age rather than size or shell thickness might be the important thing in food selection by the drills. This possibility was studied by experiments of three types.

In the first of these, individual Urosalpinx were placed with a choice of foods in compartments of a wire cage suspended just off the bottom of a tidal creek close to its outlet into Barnegat Bay. The cage was examined daily and attacks on the

shellfish were tabulated. Six Urosalpinx, each in a separate compartment, were observed with the same food choice over periods of time from 6 to 28 days. These experiments were done in the summer of 1936 so "1936 set" indicates oysters spawned and set in the current season, "1935 set" are one-year old oysters, etc. Results of the wire cage experiments are given in Table I:

TABLE I

WIRE CAGE EXPERIMENTS - CEDAR CREEK, 1936

Single Urosalpinx in Individual Compartments.

Food Choice	Drills Used	# of Determinations	# of Attacks Made	Ratio of Attacks
1936 set vs 1935 set	6	6	32 to 2	16 to 1
1935 set vs 1933 set	6	28	42 to 20	2.1 to 1
1936 set vs Mytilus	6	8	25 to 7	3.6 to 1

These wire cage experiments indicate that the drills do prefer the younger oysters but give no clue to the factors governing the choice.

The second type of experiment was designed to see whether the drills actually explored both possibilities when provided with a choice of foods. Ten Urosalpinx were placed in the center of a flat Pyrex dish and oysters of different ages were grouped in opposite corners. The drills were watched and movements recorded until they remained stationary for at least an hour. For one series, the final locations of drills that had moved to oysters are shown in Table II:

TABLE II

DISH EXPERIMENTS - 1936

Series I - Food Choice 1936 set vs 1935 set

Ten Urosalpinx in Dish with Oysters

Number of Determinations	Drills on 1936 set	Drills on 1935 set
1	1	2
2	3	1
3	1	2
4	2	1
5	3	1
6	3	0
7	3	0
8	10	0
9	10	0
Total	36	7

Ratio $\frac{\text{1936 set}}{\text{1935 set}} = 5.1$

Some few of these drills explored the entire dish and both groups of oysters, but the great majority of them moved directly to one of the groups of oysters and stayed there. This simple experiment indicates preferential orientation to some substance from the younger oysters. A choice is not made on the basis of shell thickness.

The third type of experiments was designed to establish whether or not the drills respond differently to substances given off by oysters of different ages. Bay water from an overhead tank, filled freshly at each high tide, flowed into two chambers of a double tank. Shellfish of different ages were placed in the chambers and the overflow water was led to opposite corners of a rectangular, flat-bottomed dish, in the center of which 15 drills had been placed. The Urosalpinx then oriented to overflow water alone. A series of experiments by Federighi had shown that Urosalpinx orients precisely and moves against a current of water. Consequently special precautions were taken

to see that rheotropism was not controlling the movement of the drills. The data presented in Table III are representative of the type obtained in these overflow tank experiments. When all drills in the dish remained stationary, their positions were noted. The numbers clustered around the overflow tube from the chamber containing 1936 set oysters and around the overflow tube from the 1933 oysters are shown in the Table. In the eight determinations in this series, for every drill moving to the water from the three year-old oysters, four were attracted to the water from the younger oysters.

TABLE III

OVERFLOW TANK EXPERIMENT - 1936

Series III Food Choice - 1936 set vs 1933 set

Fifteen Drills in Eight Determinations

Number of Determination	Drills at 1936 Inlet	Drills at 1933 Inlet
1	8	2
2	4	2
3	4	2
4	7	1
5	5	0
6	6	0
7	6	3
8	5	1
Total	45	11

Mean ratio $\frac{1936}{1933} = 4.1$

A variety of drill foods were compared in this way and the data obtained are summarized in Table IV. The information given in Table III is summarized here as Series III:

TABLE IV

OVERFLOW TANK EXPERIMENTS - 1936

Summary of Data

Series	Food Choice	Number of Determinations	Drills to "Young"-Old"	Drills to "Young" / Drills to "Old"
I	1936 set vs 1935 set	14	68 - 29	2.3
II	1935 set vs 1933 set	12	53 - 25	2.1
III	1936 set vs 1933 set	8	45 - 11	4.1
IV	1933 set vs 1930 set	5	14 -- 18	0.8
V	1936 set vs Mytilus	4	15 - 5	3.0 (Oyster) (Mytilus)

The first three series of experiments listed in Table IV agree in showing that water from young oysters is more attractive to the drills than water from the older oysters. Twice as many drills are attracted to effluents from the 1936 oysters as to effluents from the 1935 oysters, which are in turn, twice as attractive as effluents from the 1933 oysters. We might expect therefore that water from the 1936 oysters would attract about 4 times as many drills as the 1933 effluents. This expectation is realized in series III. Little preference is shown between the effluents from the 3 year old and the 6 year old oysters (series IV). This series indicates that the differences in effluents which enabled the drills to distinguish between young and old oysters disappeared after these oysters reached an age of about three years.

These laboratory experiments point conclusively to a dominant role of chemical attraction in food selection by Urosalpinx. In 1937 field studies on the migration rates of the drills provided additional evidence. In these field studies various groups of oysters were placed in opposite corners of a 10-foot square laid out on the sandy-mud of a Delaware Bay tide flat. Marked drills were planted in the center of the square and then were collected on the following low tide. Results of a series of these experiments are given in Table V.

TABLE V

FOOD CHOICE EXPERIMENTS - CAPE SHORE TIDE FLAT - 1937

10-foot Square on Bottom:

Series	Food Choice	Number of Drills Used	Drills on Young	Drills on Old	Drills on Young / Drills on Old
I	1936 set vs 1935 set	134	42	10	4.2
II	1936 set vs 1934 set	290	86	9	9.5
III	1936 set vs M.R.C.*	150	78	33	2.4
IV	1934 set vs M.R.C.*	1100	77	338	0.23

*M.R.C. designates a group of older oysters of indefinite age tonged from the Maurice River Cove.

These studies were done in early summer so no current season set were available. The preference ratio for 1-year-old oysters compared with 2-year-olds (series I) was 4.2. As expected from the laboratory studies, this ratio rose sharply when 1 year-olds were compared with 3 year-old oysters (series II). The decline in ratio of preference between 1 year-old oysters and the older Maurice River Cove oysters was unexpected (series III). From the results of series II and series III it was calculated that the preference ratio between 1934 set and Maurice River Cove oysters should be approximately 0.25. The check value of 0.23 obtained experimentally in series IV indicates the value of this method in measuring quantitatively the relative attraction of various shellfish for the drills.

It is of interest to speculate why the older oysters from Maurice River Cove were more attractive to the drills than the younger oysters. The three groups of younger oysters all came from an artificial oyster reef on the Cape May tide flats where these experiments were performed. This is an area where rapid growth and metabolism occur. In the Maurice River Cove areas, from which the older oysters had been tonged, growth is notoriously slow, but these oysters, when transplanted to more favorable growing grounds, frequently grow very rapidly. It is reasonable to suppose that these Maurice River oysters after transplantation to the Cape Shore, were stimulated by favorable conditions to rapid growth and high metabolism, a state characteristic of young oysters. The Urosalpinx reacted to them as though they were very young oysters.

Such studies as these described in this paper may provide valuable clues in finding more effective baits for traps used in control of the oyster drill. For example, Dr. L. A. Stauber

in large-scale trapping experiments on Delaware Bay beds in 1939 found that traps baited with old Maurice River Cove oysters caught drills more readily than those baited with young seed-sized oysters. A promising chemical approach to an improved drill bait would be the determination of the substances attracting drills to young or actively metabolizing oysters.

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Some Recent Investigations of Native Bivalve Larvae in New Jersey Estuaries

by

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The study of the larvae of marine bivalves provides information on the role played by these shellfish in the production of food for man and for other animals in the estuarine community. For example, ecological studies of the larvae of the eastern oyster (Crassostrea virginica Gmelin) have made possible prediction of the location and extent of spatfalls and the more efficient collection of seed. Research on the larvae of such commercial bivalves as the quahog (Venus mercenaria Linne), the soft shelled clam (Mya arenaria Linne), the bay scallop (Pecten irradians Lamarck), the surf clam (Spisula solidissima Dillwyn), and of the larvae of such potential food sources as the edible mussel (Mytilus edulis Linne) and the razor clam (Ensis directus Conrad) will undoubtedly lead to similar helpful results. Likewise, establishment of the precise identity of the majority of the bivalve larvae of coastal waters would permit the determination, through systematic sampling, of the kind and relative abundance of adult shellfish present in those waters in which the usual laborious dredging surveys for adults have not been made. Identification studies are also fundamental to a determination of the breeding seasons, length of larval life, degree of larval mortality, larval migrations, and dates of larval settlement. Such investigations should further stimulate investigations of the morphology, physiology, and ecology of the larvae. In the long run, these collective studies when coupled with basic research on the nutrient requirements of both the larva and of the adults may well accelerate production of desirable bivalve shellfish in our coastal waters.

At the present time, however, but little is known of the biology of the larvae of bivalves as a whole. Maxwell Smith (1945) records the total number of species of bivalves occurring in the coastal waters of middle eastern North America as 77. Of these the larvae of only seven of the commoner bivalves have been grown in the laboratory to the setting stage from parents spawned in captivity: Wells (1927) cultured the quahog, soft shelled clam, bay scallop, edible mussel, and the eastern oyster; Loosanoff (personal communication, and 1950) has recently repeated the laboratory culture of the larvae of the soft shelled clam, the quahog, and the eastern oyster, and has added the successful culture of the larvae of the surf clam to this list; T. Nelson (personal communication) has also grown the larvae of the quahog to the setting stage in the laboratory. Such culture techniques afford the only way of accurately determining the identity of native bivalve larvae.

The seven bivalves just listed are oviparous. During the spawning season they pass sex products into the water where fertilization of the eggs occurs. Fragile floating ciliated embryos soon develop which shortly grow into fully shelled though still microscopic free-swimming larvae. Natively, many of these purse-shaped larvae are able by their swimming movements to maintain recognizable vertical stratification. One of them at least, the eastern oyster, in New Jersey waters, is able in this manner to migrate toward the headwaters of estuaries during its two week larval development. At the end of a relatively short period, the period varying apparently with the kind of bivalve and with the temperature of the water, these larvae attain larval maturity and seek out a place on or in the bottom.

Two other of the 77 bivalves listed for our coastal waters, the tiny gem shell (Gemma gemma Totten) and the destructive ship worm (Teredo navalis Linne), are known to be larviparous. The gem shell retains its young in the gill chamber throughout the larval period, so that the larvae never appear swimming freely in the water. They have occasionally been taken in bottom plankton samples after storms or during very swift currents. The ship worm releases its young into the water in the early straight hinge shelled stage, after which these larvae become members of the regular planktonic community.

The larval life history of the remaining bivalves of our middle eastern coast is known only imperfectly or not all. Sullivan (1948) has recently made an excellent contribution in the study of the larval types in Malpeque Bay, P.E.I., Canada, in which she described and photomicrographed the 22 bivalve larvae regularly occurring in this water mass. She has added 13 provisional names to the list of bivalves whose larvae are free-swimming, and has set a desirable precedent in the investigation of all bivalve larval types in a single locality.

The present report is concerned principally with an investigation of the bivalve larvae of New Jersey coastal waters, principally Little Egg Harbor, where our floating laboratory, the "Cynthia", has been moored for the last three summers. The study has developed into a twofold program: first, the cataloguing of all the larvae of the estuary, identifying them where possible by reference to the scanty literature, or giving provisional names where accurate identity has not been possible. The various stages of many of the larvae have been measured, preserved in fluid and in solid media, drawn, and photomicrographed. A key to the larvae has also been constructed, which though still quite incomplete and inadequate, is something of a help in identification. The second phase of the program deals with studies of the ecology and life history of the commoner native larvae; these investigations were begun in 1938. The results of preliminary studies on the native movements of the larvae of the eastern oyster in New Jersey waters are now in

press (Carriker, 1951); and at present a study is being conducted on the ecology and life history of the larvae of the quahog, with a view to obtaining information which may lead to improved quahog production.

Recognition of the majority of the bivalve larvae in a locality minimizes the danger of misidentification of any one larva--a real danger when dealing with minute organisms of such close appearances. Misidentification is especially easy during the months of June, July, and August when after periods of bivalve spawning the larvae may appear by the thousands per hundred liters of bay water, quite eclipsing the other plankters. In fact, it was the bewildering similarity of a few of the developmental stages of some of the native larvae to comparable stages of the quahog larvae which led me to undertake a systematic survey of all of the larvae of the local estuaries. This uncertainty was encountered in spite of the fact that we had raised quahog larvae on the "Cynthia" to the early umbone stage, and that Dr. Loosanoff has kindly made available to us a very helpful series of slides of all stages of the larvae of the quahog.

In the winter months only the larvae of the edible mussel (Mytilus edulis Linne) are present in conspicuous numbers. Plankton sampling in Shark River, New Jersey, during the past two winters has disclosed the presence of all stages of mussel larvae, and conversely summer sampling in Little Egg Harbor, though mussels are numerous in the deeper channels and inlets, has never indicated their presence.

A serious obstacle facing the bivalve larvologist is the complete dearth in the literature of keys for identification of bivalve larvae. In Europe the fine beginning which Jorgensen (Thorson, 1946) has made on a study of the oldest stages of the larvae of Danish marine bivalves, and the contributions which Lebour (1938, and other years) has made on the bivalve larvae of English waters, are of considerable help. In America we have principally the very useful paper of Stafford (1912) describing and illustrating several of the commoner bivalve larvae, and the papers of Wells (1927) and of Sullivan (1948) already mentioned.

A major difficulty in larval identification arises from the fact that in their development from the purse-shaped straight hinge stage to the mature larvae, the larvae increase in size, but the dimensions generally do not increase proportionally; the color of the living larvae may change progressively with age from a glass-like appearance to a dark yellow, or brown, or purple, or mixture of these hues; and umbones of varying size and shape may appear. Color may or may not be an entirely reliable diagnostic character. Also the shades of color of some of the larvae may vary from one part of the larval season to another. The color of the digestive gland of the quahog

larva varies in intensity from a pale straw yellow to a bright orange to a greenish yellow, the color apparently varying with the type of food consumed and with the age of the larva.

On the other hand some of the larvae develop characters which are decidedly helpful in the segregation of the various species, particularly in the older stages. For example, Sullivan's quahog larva (which is a misidentification of an unknown larva) develops conspicuous dimpled markings on the umbones. The gem shell bears pronounced lattice-like patterns across the valves which distinguish the species from all others encountered. The eastern oyster and the jingle (Anomia simplex D'Orbigny) soon develop valves which are decidedly unequal, and in the jingle only one umbone is conspicuous. The ship worm larva in the later developmental stages is typically and distinctly walnut-shaped; whereas the larvae of the minute Rochefortia (Rochefortia planulata Stimpson) possess an unmistakable flattened wafer-like set of valves. And the mature stages of the eastern oyster, the ribbed mussel (Modiolus demissus Dillwyn), and the edible mussel bear characteristic eye spots on each valve.

A total of some 23 different species of bivalve larvae have been recorded for our estuaries, principally from Little Egg Harbor. Of these accurate identification has been possible for only about 10 of the species. Of the remainder final identification must await laboratory culture from known parents.

The highest concentration of bivalve larvae obtained in Little Egg Harbor this summer was pumped in late July when 80,600 total larvae per 100 liters of water were taken in a mid-vertical sample in the middle of the Harbor. These larvae were mostly of the younger stages. Older stages are never present in great abundance, indicating the high mortality which occurs during the free-swimming larval period.

Comparison of the various species and stages of bivalve larvae has been greatly facilitated by the use of a fluid preservative which has been developed over the last two years (Carriker, 1950). This consists of 1% formalin, 10% commercial sugar, and 0.05% sodium bicarbonate in filtered bay water (20-30°/oo). The pH of the fluid should be maintained at approximately 8. The larvae are killed initially in 2% neutralized formalin.

The studies of the ecology and life history of native quahog larvae have been carried out during the last three summers in Little Egg Harbor, a tidal body of water some 4 miles wide, 10 miles long, with an average depth at mean low water in the largest portion of the bay of 4 to 7 feet, and supplied by only one principal inlet, a deep narrow channel. The spring range of the tide is about 3 feet. Through some as yet undiscovered hydrographic feature the salinities of the Harbor are

unusually uniform for the entire southern two-thirds so far investigated, this summer varying during the phases of the tide cycle only about one part per thousand, away from the negligible influence of a few small creeks along the western shore. Temperatures have also been relatively uniform. Maximum current velocities vary from about 130 cm./sec. in the inlet to about 12 in the middle of the Harbor where most of the larval sampling was done. Larval studies are most productive when sampling is done at close spatial and time intervals at, at least, one central carefully selected station. Large concentrations of adult quahogs occur in the southeastern portion of the Harbor.

Observed spawning of the quahogs in Little Egg Harbor over a three year period extended at least from June 10 to September 4. Depending on water temperatures, additional observations in the spring and fall might disclose a longer spawning season. As ascertained by following quahog larval swarms from the first appearance of the straight hinge stages (when they are approximately one day old) to disappearance of the oldest stages from the plankton, and checking the size of the larval shell in recently set quahogs, the larvae grow in size from about 98 μ to 200 μ in approximately 7 days. There seems to be considerable variation in the duration of the pelagic stage, however, fastest growing larvae setting in probably as short a time as 5 days and slower growing larvae in more than 10 days. Growth of large larval swarms is fairly uniform up to 140 μ and takes about 4 days; after that the size range of the individuals within a single swarm continues to expand with age. In the three years of study the greatest concentration of quahog larvae encountered has been about 2,500 early stage larvae per 100 liters of bay water for each year, occurring in July. The oldest stages of larvae, however, become so scarce that it has been necessary to pump 500 to 1,000 liter samples to find them. Rarely are more than 5 ready-to-set larvae pumped per 100 liters of water. Mortality thus, as with all free-swimming bivalve larvae, is exceedingly high. So it would seem that normally in Little Egg Harbor quahog setting occurs over much of the summer and in relatively small concentrations--the phenomenal quahog sets of local folk lore have not yet been encountered.

Insufficient serial vertical and horizontal sampling during tidal cycles has been performed to indicate whether quahog larvae exhibit detectable migratory movements in the Harbor. Horizontally the larvae of extensive spawnings are found throughout the Harbor and at all phases of the tide cycle. Smaller spawnings may remain quite localized in a smaller mass of water and be traceable each day only by means of the phase of the tide and the depth of the water at a standard sampling station. By means of a series of periodic vertical serial samplings through the cycle of the tide it was observed that the maximum larval concentrations during daylight hours ordinarily remain about one meter depth from the surface, and that these concentrations move to a slightly higher position during maximum current

velocities. During the day the larvae seldom occur directly over the bottom. During the hours of darkness, however, preliminary sampling shows the larvae more widely distributed throughout the vertical column of water, extending to the bottom. The stratum of maximum concentration also descends. Here then is a possible response of the larvae to light, which bears further investigation.

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Growth and Setting of Larvae of *V. mercenaria* in Relation to Temperature

by

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INTRODUCTION

Almost every student of ecology or physiology of larvae of lamellibranch molluscs has commented, at some time or other, that temperature is the factor influencing the duration of their pelagic life. Unfortunately, most of these comments were usually too brief and incomplete. Others, even if lengthy, lacked supporting data to be of much value in analyzing the relationship between the temperature and growth of larvae.

Since a review of the literature devoted to the effects of temperature upon larvae of lamellibranchs has recently been offered by Thorson (1946) and Baughman (1947), there is no need to repeat it here. We shall merely mention a few articles having closest relation to our studies. J. Nelson (1908) stated that within the temperature range from 24.0 to 27.0°C. the American oyster, *Ostrea virginica*, has a free-swimming period of only one week. At 23.0°C., however, this period is extended to 13 days, and at 20.0°C., to 17 days. Medcof (1939) concluded that oyster larvae require approximately 24, 26 and 30 days to reach maturity at a constant temperature of 21.0, 20.0 and 19.0°C. respectively. According to Korringa (1941), *Ostrea edulis* of Holland has a pelagic life of six days at a temperature of 22.0 - 23.0°C., 9 to 10 days at 18.0 - 21.0°C., and 13 to 14 days at 16.0 - 17.0°C. Belding (1912) thought that the free-swimming period of larvae of *Venus mercenaria* is 10 to 12 days at a temperature of approximately 22.0°C., but somewhat longer at lower temperatures.

Thus, the general opinion is that temperature may hasten or prolong the larval period. This, of course, cannot be denied. However, since the number of days needed for larvae of different lamellibranchs to reach the setting stage at different temperatures was decided by most investigators largely upon the basis of field observations, where it was difficult and often impossible to evaluate the importance of other factors, such as salinity, pH, food, etc., the accuracy of the day-degree relationships offered may be questioned. It is thought, therefore, that such relationships can be best determined by laboratory experiments where most of the factors can be rigidly controlled. The present article offers a description of such studies devised to determine the rate of growth, length of the free-swimming period,

and of other aspects of the behavior of larvae of the hard shell clam, Venus mercenaria L., grown at different but constant temperatures.

We wish to express our appreciation to our colleague, John H. Peterson, for tabulating the data of this article.

METHODS

The larvae were grown at the constant temperatures of 33.0, 30.0, 27.0, 24.0, 21.0, 18.0 and 15.0°C. ± 1.0°C. They were kept in earthenware crocks of 20-liter capacity. These crocks, which were covered with black painted glass to exclude the light, were placed in large wooden boxes through which water of a constant temperature was running, thus maintaining a constant temperature within the crocks, which were filled with sea water filtered through thick cotton filters. Duplicate crocks were used for each temperature.

The largest part of this experiment was conducted during the winter and early spring, the time which we found most convenient to control the temperature of the water used (Loosanoff, 1949). Spawn was taken from clams which were conditioned to spawn in winter (Loosanoff and Davis, 1950). To avoid shock the fertilized eggs were gradually brought up or lowered to the temperatures in which they were to be cultured. Usually one million eggs were introduced in each crock, thus creating the initial concentration of 50,000 eggs per liter of water.

The cultures were changed every two days by using the method already described (Loosanoff and Davis, 1950). After the water had been changed the cultures were fed a mixture of Chlorella sp. and a purple sulfur bacteria of the genus Chromatium perty, creating in the culture crocks a concentration of approximately 300,000 cells of Chlorella and 400,000 cells of sulfur bacteria per cc. of water. It may be mentioned here that the sulfur bacteria, which were rejected as food by the oysters (Loosanoff, 1949a), were taken in and apparently assimilated by the larvae of V. mercenaria.

Altogether four major experiments were conducted. In the first, no larvae were measured until the fourth day. In the subsequent experiments, however, larvae from all the cultures were measured at the end of the second day after fertilization. In one 30.0°C. culture measurements were made as soon as the larvae reached the straight hinge stage, which occurred in less than 24 hours.

From each crock 50 larvae were taken for measurement on each occasion. However, because the cultures were run in duplicate, the number of larvae taken as a sample for each temperature

group was actually 100. As a rule, in all the experiments the duplicate samples showed extremely close agreement.

Measurements of the larvae were made in the usual manner, using a Sedgwick-Rafter call to hold the larvae. Since each of the small divisions of our ocular micrometer was equal to seven microns, most of our figures were based on these intervals. In some cases, however, when the necessity arose to determine the measurements more precisely, the larvae were measured under high-dry power where each division of the ocular micrometer was only 1.7 microns. In general, however, it was found impractical to be confined to such accurate measurements because the variations in the measurements of the larvae depended to some extent upon the position in which the larva was lying on the slide. This was especially true of the older larvae the shapes of which were more rounded than those of the young ones, which were relatively flat.

RESULTS

Our discussion will, at first, be confined to the temperatures within the range of 18.0 to 30.0°C. The results observed at 15.0 and 33.0°C. will be discussed later because at these temperatures the development of the eggs and larvae was usually abnormal.

Within the range from 18.0 to 30.0°C. the mortality of the eggs and then the larvae was comparatively low. It is estimated that often not less than 85 per cent of these were carried to the stage of metamorphosis. The only exceptions were several cultures grown at 18.0°C. where somewhat fewer eggs developed into larvae of straight hinge stage. However, those that reached that stage usually lived through metamorphosis.

The larvae in all cultures appeared and behaved normally. They were usually vigorous swimmers rapidly moving through the water, this condition necessitating killing them with formalin before taking their measurements. Their color was usually pinkish-yellow-green characteristic of the type of food they were fed. Incidentally, our experience has shown conclusively that the color of the larvae of V. mercenaria, as well as that of the larvae of some other lamellibranchs, such as M. arenaria and M. solidissima, with which we worked at different times, should not be considered as a reliable specific characteristic that may be helpful in identifying larvae. We formed this conclusion because we found that the color of the larvae may be changed at will, within an hour or so, by feeding them micro-organisms of different colors.

The data on the growth of the larvae at different temperatures in Experiments 1 through 4 are given in Tables 1, 2, 3, and 4. These tables give the minimum, maximum and mean sizes

TABLE I. Minimum, maximum and mean length of larvae of different ages grown at five constant temperatures. Each sample consisted of 100 larvae. Experiment 1.

DAYS	18.0°C.			21.0°C			24.0°C			27.0°C.			30.0°C.		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
4	100	121	111	100	129	117	100	143	122	107	150	134	100	171	140
6	100	136	116	107	150	130	100	157	136	107	157	143	121	200	168
8	-	-	-	107	172	144	107	164	146	114	172	148	107	214	186
10	107	164	132	121	179	158	107	172	150	121	172	151	-	-	-
12	-	-	-	121	186	164	121	193	158	129	193	156	-	-	-
14	102	197	164	136	204	171	124	197	164	124	197	168	-	-	-
16	114	212	174	121	200	172	114	200	171	136	214	176	-	-	-
18	121	214	181	121	207	182	121	207	181	150	214	187	-	-	-
20	129	221	192	136	228	196	150	221	186	-	-	-	-	-	-
22	139	228	197	157	233	198	-	-	-	-	-	-	-	-	-
24	143	221	197	-	-	-	-	-	-	-	-	-	-	-	-
26	179	229	206	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2. Minimum, maximum and mean length of larvae of different ages grown at five constant temperatures. Each sample consisted of 100 larvae. Experiment 2.

DAYS	18.0°C.			21.0°C.			24.0°C.			27.0°C.			30.0°C.		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
2	93	107	104	93	114	106	86	114	109	100	121	109	93	121	107
4	100	121	110	100	121	111	100	136	118	100	136	121	100	143	122
6	100	143	115	100	129	115	114	150	130	107	164	135	93	164	133
8	100	129	116	107	136	122	121	164	132	114	172	145	107	193	148
10	107	143	124	107	157	129	114	164	141	107	186	151	129	207	169
12	107	150	132	114	164	140	114	172	149	114	207	162	121	214	173
14	107	164	142	114	179	153	121	186	160	136	214	177	150	207	186
16	114	179	148	121	186	160	121	193	165	129	207	187	-	-	-
18	107	200	163	107	193	166	150	193	177	136	214	194	-	-	-
20	121	200	169	136	214	182	129	214	184	-	-	-	-	-	-
22	143	207	183	136	221	191	-	-	-	-	-	-	-	-	-
24	129	207	183	136	228	185	-	-	-	-	-	-	-	-	-
26	150	221	196	136	236	181	-	-	-	-	-	-	-	-	-
28	150	221	188	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 3. Minimum, maximum and mean length of larvae of different ages grown at five constant temperatures. Each sample consisted of 100 larvae. Experiment 3.

DAYS	18.0°C.			21.0°C.			24.0°C.			27.0°C.			30.0°C.		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
1	-	-	-	-	-	-	-	-	-	-	-	-	86	107	101
2	93	114	104	93	114	106	86	121	106	100	121	112	93	114	106
4	100	121	108	93	136	113	100	143	123	107	157	132	93	136	118
6	100	150	112	107	129	117	107	179	143	107	171	146	100	200	146
8	107	129	117	107	143	125	114	200	163	121	186	160	121	214	174
10	114	164	130	107	193	147	150	221	191	121	214	184	107	228	189
12	114	164	139	121	193	169	136	228	202	143	221	193	121	236	189
14	107	186	148	129	214	180	150	221	194	171	221	198	-	-	-
16	114	193	158	121	221	186	-	-	-	-	-	-	-	-	-
18	114	200	161	143	221	195	-	-	-	-	-	-	-	-	-
20	129	207	169	157	221	195	-	-	-	-	-	-	-	-	-
22	121	214	174	143	236	187	-	-	-	-	-	-	-	-	-
24	136	221	179	-	-	-	-	-	-	-	-	-	-	-	-
26	143	214	189	-	-	-	-	-	-	-	-	-	-	-	-
28	136	236	182	-	-	-	-	-	-	-	-	-	-	-	-
30	136	214	179	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 4. Minimum, maximum and mean length of larvae of different ages grown at four constant temperatures. Each sample consisted of 100 larvae. Experiment 4.

DAYS	21.0°C.			24.0°C.			27.0°C.			30.0°C.		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
2	95	117	106	95	117	107	102	124	111	102	124	110
4	102	132	117	102	146	121	109	153	129	102	168	135
6	109	146	127	109	161	136	109	190	151	117	124	170
8	109	161	133	109	182	144	132	197	159	132	226	191
10	109	175	143	109	197	160	146	212	182	146	219	191
12	117	182	152	124	204	168	124	219	192	-	-	-
14	132	190	159	124	212	176	-	-	-	-	-	-
16	139	197	163	132	212	177	-	-	-	-	-	-
18	139	204	170	139	212	178	-	-	-	-	-	-
20	146	204	175	146	204	181	-	-	-	-	-	-
22	153	204	179	-	-	-	-	-	-	-	-	-
24	161	212	184	-	-	-	-	-	-	-	-	-
26	143	212	186	-	-	-	-	-	-	-	-	-
28	153	219	189	-	-	-	-	-	-	-	-	-

of the larvae as determined by the measurements of representative samples at two-day intervals. The exception was, as already mentioned, Experiment 1 where the larvae were not measured until the end of four days, and where no measurements were made of the larvae grown at 18.0°C. on the eigth and 12th days (Table 1). In Experiment 4, which was conducted in the early summer when the water temperature was already higher than 18.0°C., no cultures were grown at that temperature.

Before proceeding with the discussion of the results of our experiments it should be remembered that metamorphosis of a larva into a young clam is not as sharply defined as is metamorphosis or, as it is commonly called, setting of an oyster where the larva ceases crawling entirely and cements itself to a shell or other clean object. In the case of V. mercenaria, as well as some other clams, metamorphosis is rather an extended process beginning with the gradual replacement of a ciliated velum with a large muscular foot and ending with the development in the foot of a functional byssal gland. This point is often difficult to establish because many young clams, although possessing a byssus gland, do not always attach. Therefore, it may be difficult at times to distinguish a recently metamorphosed clam from an old larva, especially if the animal does not move.

The length at which metamorphosis took place in our cultures ranged from approximately 175 to 236 μ, occurring most commonly between 200 and 210 μ. The largest larvae did not always metamorphose first. In several cultures some comparatively small individuals measuring only approximately 180 μ did metamorphose, while larger larvae, measuring more than 200 μ, still continued swimming, displaying a powerful velum and a comparatively small foot.

In the course of these experiments we tried to determine whether the larvae grown at low temperatures, such as 18.0°C., would reach a larger size before setting than the larvae grown at higher temperatures. Some of the preliminary experiments did indicate a tendency of this type, but further and more extensive experiments did not support this contention. So far no definite evidence is available that such a rule applies to the larvae of V. mercenaria.

The results of the first experiment showed that, with exception of the 30.0°C. group, the rates of growth of the other four temperature groups were closely resembling each other, indicating that within the temperature range of 18.0 to 27.0°C. the differences in the temperature do not always significantly affect the rate of growth (Table 1). If plotted, the data would not resemble the exponential growth curves offered by Medcof (1939) for the growth of larvae of O. virginica. Furthermore, contrary to the findings on some other lamellibranchs (Seno et al, 1926) the larvae kept at 30.0°C. did not die but showed healthy, rapid

growth, some of them reaching the setting stage during the seventh day, i.e., considerably ahead of the cultures kept at lower temperatures.

Subsequent studies (Tables 2, 3 and 4) showed that the results of Experiment 1 were somewhat atypical. Nevertheless, they are included here for the purpose of comparison and discussion, and partly to demonstrate the possible deviations that may occur in this type of study. Naturally, the explanation was sought for the peculiar results obtained. Investigation of the methods and procedure used during the experiment showed, however, that the only factors that varied from culture to culture were temperature and, perhaps, aeration. In other respects, such as number of larvae, quantity of food, salinity, pH, amount of light, etc., all the cultures were presumably subjected to identical conditions. The differences in the temperatures were, of course, the basic part of the experiment. The differences in the aeration, the only factor that was not rigidly controlled, had to be properly evaluated. The following experiment was designed to evaluate the importance of such differences.

Several cultures of larvae were started at identical conditions except the degree of aeration. Some crocks were aerated very vigorously so that the surface of the water in them resembled the action of boiling water; other crocks were aerated only feebly, while the third received no aeration whatsoever with exception of unavoidable splashes occurring during the change of water which was made every second day. The results showed that after the larvae had reached the swimming stage vigorous aeration was unnecessary. Clean, carefully attended cultures could be brought to the setting stage without continuous aeration. The larvae of the unaerated cultures showed approximately the same percentage of survival, grew at the same rate and began setting at the same time as either the feebly or strongly aerated cultures which, in turn, showed no significant differences. Obviously, since the differences in degree of aeration in the cultures of Experiment 1 were incomparably smaller than those of the specially designed experiment, we may conclude that those variations were not responsible for the differences in the rates of growth of the larvae in the different cultures of Experiment 1.

Experiments 2, 3 and 4 agreed, in general, that the rate of growth of the larvae was more rapid at high than at low temperatures (Tables 2, 3 and 4). However, even in these experiments certain discrepancies were noted. For example, in Experiment 2 the mean length of the larvae grown at 27.0°C. was, on the sixth day, somewhat greater than that of the 30.0°C. group (Table 2). In Experiment 3 the mean rate of growth of the larvae at 27.0°C. was, until the sixth day, more rapid than that of 30.0°C. (Table 3). Furthermore, even the 24.0°C. group was for some time growing more rapidly than the larvae at 30.0°C.

At the temperature of 30.0°C. setting of larvae in some experiments began as early as the seventh day after fertilization, but in others it was delayed until the ninth (Table 5). The entire population of the cultures kept at this temperature metamorphosed within five to seven days after the beginning of setting (Table 6). However, the total range in days between the beginning and the completion of setting at this temperature, as based on all four experiments, extended from the seventh to the 16th day after fertilization and covered a period of nine days (Table 5).

At the temperature of 18.0°C. the earliest beginning of setting was recorded 16 days after fertilization, and the latest, 24 days after it (Table 5). The range in days between the beginning and the completion of setting at this temperature extended from the 16th to the 30th day after fertilization, thus covering a period of 14 days. It is important that while in Experiments 1 and 3 setting at 18.0°C. extended for 12 and 11 days respectively, in Experiment 2 it was completed in only six days, thus indicating considerable variations in the behavior of the population of clam larvae kept presumably under identical conditions.

The number of days needed after fertilization for the beginning and for the completion of setting of larvae at the three intermittent temperatures of 21.0, 24.0 and 28.0°C. are also given in Table 5. With exception of Experiment 1, where setting at three different temperatures began on the same, the 14th, day, the number of days, in general, increased with a decrease in temperature. Furthermore, even in Experiment 1, the range of setting in days increased with a decrease in the temperature. Thus, for example, while at 27.0°C. the setting was completed in 20 days after fertilization, at 21.0°C. it continued until the 24th day (Table 5).

The number of days elapsing between the beginning and the end of setting in the cultures kept at different temperatures did not follow a definite pattern throughout all the experiments. Experiment 1 was the only one in which there was a definite trend showing that the number of days needed for the completion of setting of the entire larval population decreased with an increase in temperature (Table 6). In that experiment 12 days were needed to complete the setting at 18.0°C., while at 30.0°C. setting of the entire population was completed in five days. In Experiment 2, however, no such relation was found. In fact, setting was completed within a somewhat shorter period at 18.0°C. than at 30.0°C. Experiments 3 and 4 in this respect also present a somewhat inconsistent picture, although Experiment 4, in general, resembles the trend found in Experiment 1 (Table 6).

Our studies showed that the larvae, which came from the same parents and were kept under identical conditions in the same crocks, showed great variations in their size (Tables 1, 2, 3 and 4). Taking, as an example, the measurements made in

TABLE 5. Number of days needed after fertilization for the beginning and for the completion of setting of larvae at different temperatures in each of four experiments; range in days between the beginning and completion of setting, and the maximum number of days during which setting may extend in cultures grown at the same temperatures.

T.°C.	DAYS				RANGE IN DAYS	NUMBER OF DAYS
	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4		
18.0	16-28	24-30-	19-30	-	16-30	14
21.0	14-24	20-26	17-22	18-28	14-28	14
24.0	14-22	17-22	11-14	14-22	11-22	11
27.0	14-20	13-18	9-14	10-14	9-20	11
30.0	7-12	9-16	7-14	7-12	7-16	9

TABLE 6. Number of days elapsing between the beginning and the end of setting in the cultures kept at different temperatures.

T.°C.	NUMBER OF DAYS			
	Expt. No. 1	Expt. No. 2	Expt. No. 3	Expt. No. 4
18.0	12	6	11	--
21.0	10	6	5	10
24.0	8	5	3	8
27.0	6	5	5	4
30.0	5	7	7	5

Experiment 2, one will find that early in the experiment, on the second day after fertilization, the range in the length of the larvae was comparatively small (Table 2). At a temperature of 18.0°C. it ranged between 93 and 107 μ, while at 30.0°C. it extended from 93 to 121 μ. However, as the experiment progressed, the difference between the minimum and maximum sizes increased and towards the end of the experiment the length range of the larvae in the 18.0°C. culture extended from 150 to 221 μ and in the 30.0°C. culture, from 150 to 207 μ. At some intermediate temperatures, for example that of 21.0°C., the differences in the length of the larvae, during the last days of the experiment, were even greater extending from 136 to 228 μ (Table 2).

The data offered in Tables 1 to 4 inclusive gave only the extent of the larval sizes without showing how prevalent certain size-groups were in the larval populations. An example of such length-frequency distributions is given in Table 7, which is based upon the measurements of the larvae grown at 18.0, 24.0 and 30.0°C. in Experiment 2. The temperatures selected represented the lowest, the highest and the average of the five temperature classes. Experiment 2 was chosen as the example because it appeared to be most closely approaching the mean of all four experiments. At the end of two days the length-frequency distribution of the larvae in all three cultures was relatively uniform occupying a comparatively narrow range and showing a modal class of approximately 107 μ. Between the fourth and sixth days, however, the differences between the length-frequency distribution of the larval population of the three cultures had already become prominent. The differences were especially evident in the sizes of the modal classes. While for the 18.0°C. group this class at six days measured 114 μ, for the 24.0 and 30.0°C. groups these classes measured 129 and 143 μ respectively (Table 7).

As the experiment progressed, the differences in the length-frequency distribution of the larvae of the three cultures remained significant. In all instances the modal classes of the cultures grown at higher temperatures were larger than those at lower ones (Table 7).

Our data also showed that the length-frequency distributions of the larvae grown at the same temperatures but in four different experiments were not always closely resembling each other. For example, examining the length measurements of the larvae grown at a temperature of 24.0°C. in each of the four experiments, we found that the differences in the modal classes of the four cultures became apparent as early as the fourth day (Table 8). At eight days these differences became even more sharply defined. On the 12th day, while the cultures of Experiments 1 and 4 showed considerable agreement, and the culture of Experiment 2 was not greatly different from those two other cultures, the culture of Experiment 3 was definitely deviating from

TABLE 7. Length-frequency distribution (expressed in per cent) of larvae of different ages grown at temperatures of 18.0,24.0 or 30.0°C. Experiment 2.

TEMP.	18.0°C.														24.0°C.										30.0°C.						
DAYS	2	4	6	8	10	12	14	16	18	20	22	24	26	28	2	4	6	8	10	12	14	16	18	20	2	4	6	8	10	12	14
MICRONS																															
86															1																
93	2																							2		1					
100	37	1	3	1											10	1								19	1						
107	61	54	28	16	6	3	1		2						54	7								52	8	6	1				
114		43	34	44	20	3	4	2	1						35	44	8		4	2				25	22	4	1				
121		2	27	29	29	15	9	1	1	1						38	20	6	2		2	2		2	35	14	8		1		
129			7	10	22	30	7	7	1			2				8	34	22	12	8	2			2		22	18	5	1		
136					18	23	13	17	2	1						2	30	20	24	12	8			2		11	21	8	2	4	
143			1		5	20	26	19	9	6	1	2					6	42	32	16	6	4		2		1	25	16	2	4	
150						6	20	21	7	8	1	2	1	1			2	6	14	24	14	16	6	6			10	26	9	6	1
157							14	16	18	14	2	5	1	4				2	10	22	14	12	2	8				22	17	5	1
164							6	12	14	19	5	4	1	10				2	2	10	22	28	14	4			1	4	15	13	6
172								3	20	12	10	12	1	6						6	20	18	14	4				8	15	18	9
179								2	13	15	24	21	9	17							8	10	24	4					17	20	15
186									8	17	29	15	15	18							4	6	34	16					16	17	28
193									3	5	18	11	25	12								4	6	20				1	5	5	24
200									1	2	8	17	17	14										18						5	13
207											2	9	18	8										12					1	1	3
214													11	7										2						1	
221													1	3																	
228																															

TABLE 8. Length-frequency distribution (expressed in per cent) of larvae of different ages grown at a temperature of 24.0°C. in the four experiments.

EXPT.	No. 1									No. 2										No. 3							No. 4								
DAYS	4	6	8	10	12	14	16	18	20	2	4	6	8	10	12	14	16	18	20	2	4	6	8	10	12	14	2	4	6	8	10	12	14	16	18	20
MICRONS																																				
86										1										2																
93																				5							3								
100	1	1								10	1									19	2						14	1							
107	12	4	1	1						54	7									56	6	1					45	5	1	1					
114	19	1	4	1			1			35	44	8		4	2					17	21	4	1				34	24	5	3	1				
121	27	8	3		1	2	2	2			38	20	6	2		2	2			1	33	4	3				4	38	12	9	2	1			
129	28	14	4	4	1	4	1				8	34	22	12	8	2			2		17	15	1					22	16	14	2	1	2	1	
136	10	33	13	11	6	3	3	1			2	30	20	24	12	8			2		1	16	2		1			8	26	14	4	2	2	1	1
143	3	25	17	15	9	5	6	4				6	42	32	16	6	4		2		2	22	6					2	24	12	10	4	2	1	1
150		12	28	27	16	7	2	5	3			2	6	14	24	14	16	6	6			14	14	3	1	1			10	13	14	7	3	2	2	2
157		2	22	23	20	13	5	7	3				2	10	22	14	12	2	8			11	14	1		1			4	16	17	14	9	7	4	1
164			8	16	25	21	14	1	10				2	2	10	22	28	14	4			9	21	7	2	3			2	12	17	24	14	15	11	5
172				2	14	21	20	8	6						6	20	18	14	4			3	13	10	2	7				5	14	18	13	19	23	19
179					7	13	19	16	13							8	10	24	4			1	9	12	5	6				1	11	8	14	19	21	27
186						6	18	18	19							4	6	34	16				10	13	7	11					5	8	17	13	18	25
193					1	4	8	20	21								4	6	20				5	9	8	22					2	8	14	11	13	15
200						1	1	11	14										18				1	14	18	24					1	4	7	7	4	5
207								7	9										12					20	27	18						1	2	3	1	1
214									1										2					10	16	6							1	1	1	
221									1															1	11	1									
228																									2										

the others. The difference was not only in the size-range of the larvae but also in the size of the modal class which measured 207 μ, as compared with 164 μ of the modal classes in the cultures of Experiments 1 and 4.

On the basis of this comparison it may be concluded that the length-frequency distribution of the larvae of the four experiments, in which they were grown at the same temperature and kept under virtually identical conditions showed, nevertheless, considerable differences.

The question naturally arises whether, regardless of all the precautions taken, there might have been some not-easily-identifiable differences existing during the conduct of the four experiments, which could explain the certain lack of uniformity of the results. It is certain that, as far as the method and procedure are concerned, they were the same in all the experiments. However, since the experiments were not conducted simultaneously, but one after another, it is possible that the water used in the different experiments did differ in some respects. Such a possibility is, at present, merely a speculation because, as far as salinity, pH, and other presumably important factors were concerned, the water did not show any noticeable differences. Furthermore, as already mentioned, the water used in all the experiments was always filtered through a thick layer of cotton which removed most of the plankton. Some of the minute quantities of micro-plankton, however, passed through the filter. It was considered possible that the differences in the quality of this micro-plankton may have accounted for the differences noted in the four experiments.

The suggestion does not appear to be well supported when it is remembered that the quantity of micro-plankton passing through the filters would be so small that in our dense cultures of larvae it would not add appreciably to the food supply. Nevertheless, the suggestion should not be entirely disregarded, as it may be that the material passing through the filter might have been carrying traces of some substances necessary for the development of the larvae. It is also considered possible that the water itself may contain at different times some dissolved substances which, in a manner not yet understood, may affect the rate of development of bivalve larvae. This possibility merits careful study.

We shall now refer to the results obtained in the experiments in which the eggs or larvae were kept at 16.0 or 33.0°C. If recently discharged eggs were placed in water of 15.0°C. within three hours after fertilization, virtually none of them would undergo normal development resulting in the formation of straight hinge larvae. Some, however, would develop into trochophore larvae, usually of abnormal appearance.

If the eggs were kept at the room temperature of about 20.0 - 21.0°C. for three to four hours after fertilization and then placed in water of a temperature of 15.0°C., a few would develop into straight hinge larvae. However, the majority of these larvae would be abnormal and soon die.

Fertilized eggs kept at room temperature for six or nine hours before being subjected to a temperature of 15.0°C. gave a greater number of individuals reaching the straight hinge larval stage, but the majority developing that far would show signs of abnormal development and soon perish.

If the eggs and the resulting larvae were kept at room temperature for two days, until the straight hinge stage was well formed, and the larvae were then placed in water of 15.0°C., many of these larvae would survive. Regardless of slow growth it is possible that, under certain conditions, some of these larvae will reach the stage of metamorphosis.

This experiment demonstrated rather conclusively that the eggs of V. mercenaria in early stages of cleavage require for their normal development and survival a somewhat higher temperature than the eggs or larvae in later stages. The experiment also suggested that it is highly improbable that under natural conditions V. mercenaria would discharge eggs at a temperature of 15.0°C. ± 1.0°C. because the eggs discharged at such a low temperature would not be able to develop normally.

Our results in culturing eggs and larvae of V. mercenaria at a relatively low temperature are in close agreement with those of Seno et al (1926) who found that only a few of the eggs of Ostrea gigas kept at about 16.0°C. developed into shelled larvae most of which would be abnormal and soon die. At about 14.0°C., however, the segmentation of the eggs was so abnormal that none developed into straight hinge larvae.

Practically the same results as those recorded at 15.0°C. were again observed at the other end of our temperature range - at 33.0°C. An abnormal development and heavy mortality occurred if fertilized eggs were immediately transferred to water of a temperature of 33.0°C. ± 1.0°C. However, as is the case of the 15.0°C. observations, if the eggs were allowed to develop at room temperature for 48 hours and then transferred to water of a temperature of 33.0°C., a rapid, normal development followed resembling that noticed in the cultures kept at 30.0°C. In general, these observations, as well as those made at 15.0°C., fully support the point of view expressed many years ago by Pelseneer (1901) that young cleavage stages of molluscan eggs occur within a narrower temperature range than the later stages.

In the course of our experiments many thousand measurements were made of the length and width of the larvae of different ages and sizes. The size of the smallest straight hinge larvae

found in our cultures was 86 x 64 μ. Regardless of the extremely varied ecological conditions under which these and many other experiments, not discussed in this article, were conducted, we have never found a larva measuring in length more than 240 μ. This makes it highly improbable that, as reported by Stafford (1912), the larvae of V. mercenaria may grow up to 448.5 μ long. Furthermore, a comparison of the shapes and dimensions of the larvae of different sizes, which Stafford assumes to be V. mercenaria, with those of larvae grown from the eggs of adult clams made to spawn in our laboratory (Loosanoff and Davis, 1950) shows beyond all doubt that Stafford (1912) mistook the larvae of some other bivalves for those of V. mercenaria.

Similar remarks may be made about the conclusions of Sullivan (1948) that the larvae of V. mercenaria may reach the size of 320 μ. However, in a recent letter to one of us Miss Sullivan writes as follows:

"It seems likely then that I have confused the larvae of P. morrhuana and V. mercenaria. This mistake is attributable to the fact that the newly settled spat of both types were very scarce in my collections. It was, furthermore, difficult to distinguish between the two types of very young spat or to identify either type with certainty.

"If it is the case then that my larva, P. morrhuana, is really V. mercenaria, it follows that the settling size of Venus larvae in Malpeque Bay corresponds reasonably well with that of Venus larvae in your cultures."

Incidentally the disagreement between Sullivan and us on the general characteristics of the larvae of V. mercenaria extends also to the color of the larvae. Sullivan (1948) considers the color as a distinctive feature which she uses for identification of larvae of lamellibranchs. Actually, as already mentioned, we found that the color of larvae in any stage of development could be easily changed by feeding the larvae different types of food. Therefore, in nature, where the predominating species of micro-plankton may change daily, correspondingly frequent changes in the color of the larvae may be expected.

In concluding the article we would like to incorporate in it some of the observations which, we think, are of importance in studies of the growth and development of clam larvae. The first of these deals with the relative merits of detritus as food of larvae. As is generally known, during the past few years there has been a strong movement in biological circles to prove that detritus was the principal food of lamellibranchs (Coe, 1948). To determine the relative importance of detritus as food of larvae of V. mercenaria experiments were designed in which some cultures of larvae were fed Chlorella, others, a mixture of Chlorella and sulfur bacteria, and still others were

given a large quantity of detritus of one or two types. Type 1 consisted of detritus collected from the bottom of the large tank in which plankton was grown for eight months previous to collection of the sample. During that long period, of course, many generations of plankton died and fell on the bottom creating a heavy layer of detritus which measured in excess of ¼-inch. This material was collected, filtered to remove large particles which could not be utilized by the larvae and then fed to the larval cultures.

The second type of detritus was obtained during the low tidal stages from the bottom of the tidal pools formed on the tidal flats. This detritus was also filtered to remove the large particles, and then fed to the larvae.

The results of these experiments showed that there was no evidence, whatsoever, to support the contention that the larvae of V. mercenaria would grow better on detritus than on living Chlorella, or on a mixture of living Chlorella and sulfur bacteria. The cultures grown on detritus were always much inferior to those fed with Chlorella or its mixtures with bacteria. As a rule, detritus-fed cultures often died from starvation before the larvae reached the stage of metamorphosis.

The second observation concerns the density of the larval population in the cultures. As already mentioned, our experiments usually began with approximately 50 eggs per cc. of water. This was a much heavier concentration than practiced or advocated by other investigators who usually emphasized the danger of overcrowding the larvae. However, as a matter of fact, we succeeded in growing to metamorphosis cultures of V. mercenaria containing more than 100 larvae per cc. of water. Our concentrations, therefore, may appear to those workers to be unrealistically dense. However, since cultures of these densities are carried at our laboratory to the stage of metamorphosis as a matter of routine, we think that in some instances the danger of overcrowding larvae of some species of lamellibranchs may not be as acute as believed. Apparently, in properly kept cultures a great majority of the larvae can survive such overcrowding, display a normal rate of growth and reach the setting stage in the same time as larvae of much less populated cultures.

CONCLUSIONS

Our experiments have shown that larvae of V. mercenaria may be grown from eggs within a temperature range of 18.0 - 30.0°C. ± 1.0°C. Within this range small variations in temperature, such as one or two degrees, are not as important as it was thought previously.

Although it is clear that, in general, the rate of growth of larvae decreases with a decrease in the temperature of the surrounding water, nevertheless, it appears that a combination of several factors may be more important in determining the length of the larval period than considerable differences in temperature. For example, in one of our recent experiments the largest part of the eggs obtained from the spawning of adult clams was placed in an outdoor tank, while the smallest was cultured in the laboratory in a regular hatching crock. At the beginning of the experiment the temperature in the tank was about 19.0°C. After ten days, towards the end of the experiment, the temperature of the tank water showed a slow increase approaching approximately 22.0°C. by the 14th day. The temperature of the laboratory hatching crock was, on the other hand, quite steadily maintained throughout the experiment near 24.0°C. Yet, regardless of the lower temperature in the tank, which, during the first ten days or so, was approximately 5.0° lower than that of the hatching crock, the larvae there began to set on the 14th day, while the first set in the culture crock was observed four days later. Obviously regardless of the lower temperature in the tank the general combination of the factors there was more favorable for the growth of larvae than in the laboratory culture crock.

SUMMARY

1 - Larvae of V. mercenaria were grown to metamorphosis in four experiments at the constant temperatures of 30.0, 27.0, 24.0, 21.0, 18.0°C. ± 1.0°C. Observations on development of eggs and growth of larvae were also performed at 33.0 and 15.0°C. ± 1.0°C.

2 - The size of the smallest larvae found was 86 x 64 µ, and the largest, 236 x 228 µ.

3 - Within the temperature range of 18.0 to 30.0°C. ± 1.0°C. the rate of growth of larvae was generally, but not always, more rapid at high than at low temperatures. Small differences in the temperature, such as one or two degrees, or sometimes even more, were not as important in affecting the rate of growth as was previously thought.

4 - At the temperature of 30.0°C. setting of larvae began in some experiments as early as the seventh day after fertilization. Setting of the entire larval population kept at this temperature was accomplished within five to seven days after its beginning.

5 - At the temperature of 18.0°C. the earliest beginning of setting was recorded 16 days after fertilization, and the

latest, 24 days after it. At other intermittent temperatures setting of larvae was within the bounds indicated by the two extremes.

6 - The length at which metamorphosis took place ranged from approximately 175 to 236 μ, occurring most commonly between 200 and 210 μ. There was no indication that the larvae grown at lower temperatures were reaching larger size before setting than those grown at higher temperatures.

7 - Larvae which came from the same source and were kept under identical conditions showed great variation in the sizes. In some instances the length of the larvae in an old culture ranged from about 100 μ to that of full grown larvae measuring in excess of 200 μ.

8 - The length-frequency distribution of larvae grown at the same temperature but in four different experiments showed considerable variations. These variations were found in the range of larval lengths and also in the lengths of the modal classes.

9 - If, immediately after fertilization, the eggs of clams were placed in water of a termperaure of 15.0°C. ± 1.0°C., virtually none of them would undergo normal development resulting in the straight hinge stage. However, if the eggs were kept at room temperature for nine hours before being subjected to the above temperature, some of them would reach the straight hinge stage.

10 - Eggs placed soon after fertilization in water of 33.0°C. ± 1.0°C. would show abnormal development and heavy mortality. However, if after fertilization the zygotes were kept at room temperature of about 22.0°C. for about two days and then transferred to the higher temperature, a rapid normal development of larvae would follow resulting in heavy setting.

11 - In general, the experiment showed that young cleavage stages of clam eggs occur within a narrower temperature range than the later stages.

12 - The color should not be considered as a distinctive feature to be used in the identification of lamellibranch larvae because it easily changes depending on the color of the food organisms eaten by the larvae.

13 - Auxiliary experiments showed no evidence whatsoever to support the contention that organic detritus is a better food for larvae than living phytoplankton, such as composed largely of Chlorella sp. Two types of detritus, one composed mostly of dying and decomposing plankton grown under laboratory conditions, and the other collected from the bottom of the tidal pools, were fed to the larvae, but caused their slow starvation and death.

14 - The degree of aeration was found to be unimportant in affecting the rate of survival and rate of growth of clam larvae. Clean, carefully attended cultures could be brought to the setting stage without continuous aeration.

15 - Overcrowding of larval cultures of V. mercenaria is not too easily achieved. Our cultures, as a rule, contained 50 larvae per cc. of water, and several cultures, in which the concentration of larvae was more than 100 per cc. of water, were grown to metamorphosis.

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